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THE CHEMICAL CONSTITUTION OF THE TUBERCLE BACILLUS.

By WILLIAM BULLOCH, M.D.,
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AND J. J. R. MACLEOD, M.B.,
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SHORTLY after Koch's discovery of the tubercle bacillus Ehrlich⁽¹⁾ made the important observation that this micro-organism can be distinguished from others by certain tinctorial characteristics, of which the most remarkable is its resistance, when once stained, to the decolorising action of mineral acids. This acid-resisting property or "acid-fastness" has since Ehrlich's discovery been used for the microscopic diagnosis of the bacillus. In his original communication Ehrlich recommended the addition of anilin water to the stain (fuchsin), the decolorising action being subsequently carried out by a 33% solution of nitric acid. With slight modifications, viz. the substitution of anilin water by carbolic acid and of the nitric acid by 25% sulphuric acid, the method is universally known at the present day as that of Ziehl-Neelsen^(2 and 3).

That this acid-fastness of the tubercle bacillus and its congeners is due to some peculiarity in its chemical constitution can scarcely be doubted, and attempts have not been wanting to isolate the substance.

In general it has been stated that the essential substance, in virtue of which the bacillus is acid-fast, is *fat*, and various researches have in reality shown that in the bodies of the bacilli there are considerable quantities of fat and fatty acid, and that the amounts of these constitute a peculiarity of the bacillus.

Hammerschlag⁽⁴⁾ for instance found 27% of substance soluble in alcohol and ether.

De Schweinitz and Dorset⁽⁵⁾ found the amount of fat even higher, viz. 37%. On saponifying the fat and finally decomposing with sulphuric

acid they demonstrated the existence of palmetic, arachidic and lauric acids. Klebs⁽⁶⁾ found in the pure ethereal extract a solid reddish fat melting at 42° C. and constituting 20·5 % by weight of the bacillary mass. In addition he found a colourless fat insoluble in ether, but soluble in benzene, and having a melting-point over 50° C. This fat was present to the extent of 1·4 %. By Klebs the acid-fastness is referred to these fats.

Ruppel⁽⁷⁾⁽⁸⁾ described three kinds of fatty substance which can be successively extracted by cold alcohol, hot alcohol, and by ether. Cold alcohol extracted about 8 % of the total weight of the body substance of the bacillus. On evaporation a quantity of free fatty acid was found and a fat which was readily saponified. By hot alcohol a peculiar colourless mass was obtained which began to liquefy at 65° C. but it did not become perfectly clear when heated even to 200° C. This substance was very difficult to saponify but appeared to be composed of fatty acid, esters, and higher alcohols. Further extraction of the bacilli by ether yielded a substance melting at 65°—70° C. The total amount of substances extracted by alcohol and by ether varied from a minimum of 8—10 %, to a maximum of 26 % of the total weight of the bacilli.

Aronson⁽⁹⁾ extracted the dried bacilli with an alcohol-ether mixture containing 1 % hydrochloric acid. After evaporating off the alcohol-ether a brown mass was obtained amounting to 20—25 % of the original T. B. An analysis of this substance showed it to contain 17 % of free fatty acid, the remainder consisting of a body which Aronson supposed to be of the nature of a *wax*. On prolonged boiling with alcoholic potash the wax yielded an insoluble residue which dissolved in acetic anhydride to form an acetate, showing that it contained a hydroxyl group and was probably an alcohol.

De Giaxa⁽¹⁰⁾ obtained 35·2—40·4 % by weight of substances soluble in alcohol and ether. Levene⁽¹¹⁾ obtained 31·56 % of fatty substances.

Recently Kresling⁽¹²⁾ has investigated more closely the nature of the fatty substances in the tubercle bacillus. He found that the best extractive was chloroform, the substance extracted by this reagent possessing the following properties :

Melting point	46° C.
Acid value	23·08.
Reichert Meissl value	2·007.
Hehner's value	74·236.
Saponification value	60·70.
Ether value	36·62.
Hübl's iodine value	9·92.

According to Kresling the so-called "fat" of the tubercle bacillus is a mixture of neutral fat, free fatty acid, esters, and higher alcohols (lecithin and cholesterin) and a number of extractives soluble in ether, alcohol, chloroform, and benzene. A quantitative estimation (calculated from the above values) of the fat substance soluble in chloroform showed,

Free fatty acid	14.38 %.
Neutral fats and fatty acid esters	77.25 "
Alcohols obtained from fatty acid esters	39.10 "
Lecithin	0.16 "
Substances directly soluble in water	0.73 "
Substances (soluble in water) formed during the complete saponification of the fat	25.764 %.

By treating a solution of the fat in benzene with sodium alcoholate Kresling succeeded after three days in producing saponification, and on removal of the soap and evaporation of the filtrate he obtained 380 grm. of an alcohol the melting-point of which was 43.5—44° C.

The results which we have to record are part of a general research on the chemical constitution of the tubercle bacillus. We have thought it of importance however to deal with the subject in parts, especially as the results which we have obtained in connection with the acid-fast substance complete the observations of Kresling.

Before proceeding to detail these results we have to express our sincerest thanks to Professor Bang and Dr Striboldt of Copenhagen and Professor O. Malm of Christiania for having placed at our disposal large quantities of tubercle bacilli necessary for the examination. We also desire to thank Dr J. Lewkowitsch for most valuable advice and assistance, especially in the determination of the purity of the substances which we have been able to extract.

Altogether we have experimented on several kilogrammes of bacilli—the deposit obtained by filtering autoclaved cultures for the preparation of tuberculin (T.O.)

The research has been mainly chemical, but all the products were tested for acid-fast properties by the usual methods.

From the experiments of Aronson and others it was known that the acid-fast substance is more or less extractable by substances which dissolve fats. After a long series of tentative experiments we adopted the following methods:

A weighed quantity of tubercle bacilli dried to constant weight was extracted in a 2-litre flask connected with a vertical condenser, the extracting substances being,

- (1) Methylated spirit followed by Aronson's mixture (alcohol-ether + 1 % HCl).
- (2) Methylated spirit followed by benzene, chloroform, or petroleum ether.
- (3) Aronson's mixture alone.

In each case the extraction was allowed to proceed over a water-bath for at least several hours; in some cases for several days. As a result it was found that although methylated spirit was allowed to act for days it was unable to completely deprive the bacillus of its acid-fastness. When the extract was filtered through a hot funnel a white precipitate deposited on cooling. This white precipitate was acid-fast, the acid-fast substance being therefore partly soluble in spirit.

1. On applying Aronson's mixture to the bacilli which had thus been treated with spirit a further extract was obtained which on filtration deposited a white acid-fast precipitate. This process had to be repeated six or ten times before the bacillus was completely deprived of its acid-fast properties.

2. In a second series of experiments the white acid-fast substance was obtained by extracting with benzene, chloroform, or petroleum ether as a substitute for Aronson's mixture.

3. By using Aronson's mixture from the commencement large quantities of the acid-fast precipitate were obtained.

In this way filtrates and acid-fast precipitates were collected.

Filtrates. The clear filtrates which reacted strongly acid to phenolphthalein were evaporated slowly to dryness and treated with sodium carbonate solution in the boiling water-bath and again evaporated. The resulting dry mass was then shaken up in a separating funnel with a mixture of ether and water until the ether extracted no more. In this way we obtained an ethereal extract I, and a watery extract II, the latter containing the fats in the form of soap.

I. *Ethereal Extract.* On evaporating to dryness this yielded a residue with a peculiar odour, and which presented the following reactions:

- (1) It was *not* acid-fast.
- (2) It was blackened by osmic acid.
- (3) It was readily stained by Sudan III, Fettponceau, and Indophenol, in 70 % alcoholic solutions.
- (4) Acrolein reaction positive.
- (5) It did not contain phosphorus.

(6) No cholesterin crystals were found microscopically, although Salkowski's reaction was positive.

The iodine value obtained by a modified Hübl's method¹ showed in two samples 40·8% and 38·7% respectively. From this result the percentage of olein was calculated by Hübl's formula² to be 47·3% and 44·9%. 3·2 grms. of the fat were saponified by alcoholic potash and the resulting soap decomposed by H_2SO_4 . The mixture of fatty acids obtained was semisolid at ordinary temperatures, and the solids were then separated from the fluid fatty acids by the following method:

The mass was melted in a beaker and dissolved in 70% alcohol. It was then filtered hot and allowed to cool. The solid acids separating out as a crystalline mass, were removed by filtration, and the precipitate thoroughly washed with 70% alcohol. In this way a filtrate and a precipitate were obtained:

α. The filtrate containing the liquid fatty acids was evaporated to dryness at a low temperature, and the residue was found to be instantly blackened by osmic acid and was probably oleic acid.

β. The precipitate was separated by fractional precipitation by means of an alcoholic solution of lead acetate, the exact technique being that described in Salkowski's *Practicum*.

The melting-points were determined in a capillary tube tied to the bulb of a thermometer, the latter being placed in a test-tube which was immersed in a flask containing conc. sulphuric acid.

The fractions gave the following melting-points:

- (1) 57° C.
- (2) 54·5° C.
- (3) not determined.
- (4) " "

From these results a comparison with standard melting-points shows that the acids are probably isocetinic (55° C.) and myristinic (53·8° C.).

These solid acids were not blackened by osmic acid and were not in the slightest degree acid-fast.

II. *Watery Extract.* The watery solution (from the original filtrate which had been shaken up with a mixture of ether and water) was decomposed by sulphuric acid in the heat, when a precipitate settled out. No fatty acid however collected on the surface. In order to

¹ Benedikt und Ulzer, *Analyse der Fette*, Berlin, 1897, p. 150.

² Benedikt, *l.c.* p. 173.

obtain the fatty acid the decomposed fluid was shaken with ether in a separator till no more dissolved out. The ether was then evaporated to dryness when fatty acid remained. When collected in quantity this fatty acid had a melting-point of 41°C . It was not acid-fast. As the product was doubtfully pure we are unable to say what it was, but its melting-point corresponded to lauric acid.

Besides these acids the filtrate contained lipochromes, a solution of which in chloroform gave a very distinct band at β , a little to the right of Fraunhofer's line *E*. An aromatic body was also found, but was not investigated further.

The acid-fast white precipitates. The precipitates obtained by methylated spirit as an extractor were different from those obtained by extraction with Aronson's mixture, ether, etc., although they both agreed in being acid-fast.

Precipitate from methylated spirit extract. This became syrupy at 117°C . and on heating further became black and gave off a pungent vapour. Even when twice purified by solution in spirit and filtration this precipitate still showed the same peculiarities. On heating the purified precipitate with Aronson's mixture under a condenser and filtering, a white precipitate settled out of the filtrate on cooling. This precipitate had a melting-point of 47°C ., this also being the melting-point of the substance obtained by extraction of T.B. with Aronson's mixture, benzene, petroleum ether, or chloroform. The whole purified methylated spirit precipitate was, however, not soluble in these fluids, as a darkly coloured substance still remained. This substance, strange to say, was still acid-fast.

With the investigation of this insoluble substance we have not proceeded, and we are quite in the dark as to its relation with the other acid-fast white precipitates obtained by chloroform, etc., although we believe its acid-fastness to be due to adherent wax.

As stated above the precipitate obtained by Aronson's mixture, chloroform, etc., melted at 47°C . and had the following properties:

It was intensely acid-fast. On staining with hot anilin-water, fuchsin, or carbol fuchsin for 5 minutes, it still retained its stain unaltered after immersion in 25% sulphuric acid for *eight* days. Even fuming nitric acid required several minutes to remove the stain. Counter-staining with alcoholic solution of methylene blue did not displace the fuchsin.

There can be no doubt that this substance is the chief ingredient in the bacillus which gives the latter its peculiar staining properties,

and its isolation in a state of purity was therefore a matter of considerable interest.

Saponification was attempted by dissolving it in warm petroleum ether, or in warm benzene, and adding absolute alcohol to the resulting solution until the white precipitate which formed on the first addition had redissolved and an opalescent solution had been obtained. Pieces of metallic sodium were then dropped into the solution and kept in motion by means of a glass rod until they had dissolved, only one or two pieces being added at a time. The sodium was added until no more effervescence ensued, after which the mixture was heated under a condenser for 3—4 hours.

The solution was then allowed to stand in a corked Erlenmeyer flask overnight, when a more or less copious precipitate of soap had fallen down. This was filtered by suction and the precipitate thoroughly washed with petroleum ether, and then removed to a flat dish and allowed to stand exposed to the air to remove the petroleum ether.

The filtrate poured into a shallow basin was allowed to slowly evaporate in air. In this way a white powder was obtained consisting of the alcohol mixed with sodium ethylate and sodium hydrate. This powder was partially soluble in boiling water, in which it formed an opalescent solution. To separate the alcohol the powder was placed in a large separating funnel and mixed with about 500 c.c. of methylated ether and about 100 c.c. of water. On shaking, a milky solution was obtained which reacted strongly alkaline to litmus. A 10% solution of sulphuric acid was added till the reaction became distinctly acid, when the ether at once became clear. After standing a few moments the ether was run off and allowed to evaporate in the air, when a white flaky powder was obtained. This however still contained 7% of ash, so that the above process had to be repeated till an ash-free product was obtained.

On examination the ash-free powder was found to be intensely acid and alcohol-fast. It was examined by Dr J. Lewkowitsch, who found it to give the following values :

Iodine value	9.39%.
Saponification value	49.40 "
Melting-point	44.4 "
Increase of weight on acetylating	1.2 "
Saponification value of acetylated product	69.0 "

From these results it was evident that complete saponification of the wax had not been effected. At the same time the increase of weight on

acetylating showed that a large amount of a body containing a hydroxyl group (an alcohol) was present.

Saponification of the wax by metallic sodium was therefore unsuitable in obtaining the pure alcohol, and further attempts were made with alcoholic potash, the method we have ultimately adopted being the following:

Dried tubercle bacilli were extracted with boiling methylated spirit until the bulk of the fatty substances had been removed. The remainder, which was still acid-fast, was then boiled under a condenser with alcoholic potash for several hours, cooled and allowed to stand, when the alcohol was decanted off. The residue was then repeatedly shaken with petroleum ether and the extract was allowed to evaporate, when a white residue was obtained. This was again saponified by alcoholic potash. At this stage of the process we have found that certain precautions must be taken in order to obtain a pure preparation of the alcohol. The white mass was shaken up with boiling absolute alcohol and the resulting solution filtered through a hot funnel.

From the filtrate on cooling a white precipitate separates out, to disappear again on warming. Great care must be taken that the alcoholic solution becomes entirely clear on warming, as in the residue from the petroleum ether there is frequently a certain amount of a gummy like substance insoluble in warm absolute alcohol.

Pure caustic potash dissolved in as little water as possible was then added to the alcoholic solution in the amount of 3%, and the flask placed on a boiling water-bath and connected with a reflux condenser. It was allowed to boil for 4—5 hours, then cooled and the contents removed to a large separating funnel and thoroughly shaken with petroleum ether. Extraction with petroleum ether was continued until on evaporation of the ether no residue was obtained.

The ethereal extracts were then repeatedly shaken up with distilled water in the separator until the washings were no longer alkaline to litmus. To attain this end it is necessary to shake and wash for several days.

The washed petroleum ether extract was then evaporated and the alcohol obtained as a white flaky powder.

The saponification value showed that this powder was the *pure* alcohol of the tubercle bacillus, and it was further found that it is the alcohol which gives the tubercle bacillus its acid- and alcohol-fastness; when small particles of the alcohol spread on a slide were stained with carbol fuchsin the stain was found to remain unaltered after prolonged

immersion in equal parts of 50% HNO_3 in methylated spirit and then in spirit. The fatty acids separated from the wax were not acid- or alcohol-fast, the fuchsin being instantly discharged, even with weak acid.

We have now obtained about 1 gramme of pure alcohol, and hope to be able to determine its elementary composition and molecular weight shortly.

Summary of Results.

1. Dried tubercle bacilli extracted with hot solutions of spirit, alcohol, alcohol-ether, Aronson's mixture, yield large percentages of fatty substances.

2. On filtering the boiling extracts a white acid-fast precipitate deposits on cooling.

3. The filtrates on evaporation can be saponified by soda, and on subsequent agitation with ether and water two extracts can be obtained—an ethereal and a watery extract.

4. The ethereal extract contains fat which is *not* acid-fast.

5. By saponifying the dried ethereal extract with alcoholic potash and decomposing the resulting soaps with sulphuric acid a mixture of fatty acids is obtained containing probably oleic, isocetinic and myristinic acids. None of these are acid-fast.

6. The watery extract (soap) on decomposition yielded a fatty acid with a melting point corresponding to lauric acid.

7. The filtrates also yield lipochromes to which the cultures of tubercle bacillus owe their colour.

8. The white acid-fast precipitate obtained by the original extraction can be saponified, but with great difficulty.

9. By prolonged boiling with alcoholic potash the acid-fast precipitate is decomposed and results in the deposition of an acid-fast snow-white flaky powder, and a non-acid-fast filtrate of fatty acids.

10. The chemical examination of the white flaky powder shows it to be an alcohol.

11. Acid- and alcohol-fastness of the tubercle bacillus is due to the presence of an alcohol.

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FURTHER REMARKS ON THE PRODUCTION OF A
MALARIAL FORM OF SOUTH AFRICAN HORSE-SICKNESS.

(Plate I. and Nine Temperature Charts.)

By ALEXANDER EDINGTON, M.D., F.R.S.E.,

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DURING my earlier experiments in connection with Horse-sickness I showed that donkeys could be inoculated with the virulent blood of horses dying with the disease without being seriously affected thereby¹.

Also the remarkable fact was demonstrated that the blood of such donkeys, drawn about the tenth or eleventh day subsequent to inoculation, was capable of setting up, in clean horses into which it was injected, a peculiar fever. This fever differed from the rapid sustained pyrexia of ordinary horse-sickness in having definitely marked remissions and intermissions, and in being, in such cases, usually non-fatal.

At this period—after several years' close and continued examination of the blood of affected horses—I had left off systematic examination of the blood, but in the blood of two horses in which this form of fever had been produced, I found one or two of the blood corpuscles to be infected with a parasite having some resemblance to that of Texas Fever, and I was led to think that the febrile attack had lowered the animal's resistance, thus permitting it to acquire an infection of Texas Fever to which horses are ordinarily insusceptible.

Nevertheless, I was never satisfied with this explanation, while the peculiar type of fever, so different from that found in horse-sickness, eventually induced me to make further enquiries.

It has been already shown in the former communication that when

¹ *Proc. Royal Society.*

a number of donkeys are infected with equal doses of virulent blood, the blood of one may kill a clean horse, that of another may induce a milder form of fever with remissions and intermissions which may extend over many weeks, while the blood of another may show no effect at all when inoculated into a horse. Further, it was noted that the behaviour of the temperature in inoculated donkeys gave little or no indication as to the power of infection which the blood might possess. Two hypotheses might be advanced for this; firstly, a different susceptibility of the horses which, as a matter of fact, I have found to be correct by inoculating a number of clean animals from one donkey. The second hypothesis would be to credit individual donkeys with different degrees of susceptibility and to admit that the more susceptible animal would furnish the more virulent blood. This also has been proved to hold good. A striking corollary to this is shown in the first experiment of Colonel Joshua Nunn, A.V.D., F.R.C.V.S., who on beginning his investigations at Natal in 1888 inoculated three mules with the blood of a dead one. Two of these animals showed no results, and while the third one died, he was led to conclude that death must have been due to natural infection and that the disease was not communicable. I was for a long time unable to explain Colonel Nunn's failure to infect the other two mules, but experience has shown me, while horses are highly susceptible and donkeys refractory, that mules occupy an intermediate position, and as the refractory donkey lowers the virulence of horse-sickness passed through it, even for the susceptible horse, it has to be admitted that the mule may be expected to lower the virulence for one of its own kind.

I therefore determined upon using animals, for the production of infective blood, whose refractory condition should be more or less uniform. To this end I made use of a number of "salted horses," *i.e.* horses which had passed through the virulent stage of horse-sickness and thereafter been reinoculated at recurring periods with progressively increasing doses of virulent blood, of which the maximum was never greater than 500 c.c.

The "salted" horses which I used were each inoculated once a month during the previous six months with 10 c.c. of virulent blood. Ten days after the last injection they were bled and the blood was used to inoculate clean horses by subcutaneous injection with 20 c.c.

These experiments were not made *en masse* but extended over a long period of time, from the 14th of November, 1901, to April, 1902.

The following table sets forth briefly the results obtained.

Inoculation	Horse	Salted horse from which blood was used	Date	Result
1st.	No. 1.	A	November 14.	Severe fever.
	No. 2.	A	"	"
2nd.	No. 1.	B	December 29.	Little or no fever.
	No. 2.	C	"	"
1st.	No. 3.	B	November 14.	Slight fever.
	No. 4.	B	"	Severe fever.
2nd.	No. 3.	Removed for other experiments.		
	No. 4.	D	December 29.	Slight fever.
1st.	No. 5.	C	December 4.	Severe fever.
2nd.	No. 5.	C	" 29.	Slight fever.
1st.	No. 6.	D	December 4.	Severe fever.
2nd.	No. 6.	C	" 29.	Slight fever.
1st.	No. 7.	A	December 6.	Slight fever.
2nd.	No. 7.	D	" 29.	More severe fever.
1st.	No. 8.	B	December 6.	Severe fever.
2nd.	No. 8.	C	" 29.	More severe fever.
1st.	No. 9.	A	December 19.	Mild fever.
2nd.	No. 9.	A	January 19.	Severe fever.
1st.	No. 10.	B	December 29.	Mild fever.
2nd.	No. 10.	A	January 19.	Slight attacks at intervals, culminating in a severe one at the 70th day, from which animal died of typical horse-sickness.
1st.	No. 11.	B	December 29.	Severe fever.
2nd.	No. 11.	A	January 19.	Mild fever.
1st.	No. 12.	B	December 29.	Mild fever.
2nd.	No. 12.	A	January 19.	"
1st.	No. 13.	B	December 29.	Severe fever.
2nd.	No. 13.	A	January 19.	Very severe fever.
1st.	No. 14.	C	December 29.	Mild fever.
2nd.	No. 14.	A	January 19.	Severe fever.
1st.	No. 15.	C	December 29.	Very severe fever.
2nd.	No. 15.	A	January 19.	Severe fever.
1st.	No. 16.	C	December 29.	Fairly severe fever.
2nd.	No. 16.	A	January 19.	Severe fever.
1st.	No. 17.	C	December 29.	Very severe fever.
2nd.	No. 17.	A	January 19.	"
1st.	No. 18.	D	December 29.	Very severe fever, ending in death.
1st.	No. 19.	D	December 29.	Very severe fever.
2nd.	No. 19.	A	January 19.	"
1st.	No. 20.	D	December 29.	Mild fever.
2nd.	No. 20.	A	January 19.	"
1st.	No. 21.	E	January 31.	Mild fever.
2nd.	No. 21.	F	February 24.	"

These twenty-one animals were obtained from the military authorities and were believed to have been recently imported. Of the two last, however, one (a stallion) was found to have been bred in the colony, while No. 20 had been certainly in the country for some time and in use during the war.

Five additional animals were subsequently obtained in the Albany district and experimented upon in the same manner.

During the periods shown, "control" animals were kept in the same stables, their stalls being frequently changed for those previously filled by animals suffering from the fever.

As a result nearly all the inoculated animals developed the fever, but those obtained locally showed it least of all.

During the febrile periods well-defined malarial parasites were found within the red blood corpuscles. In many cases the infected corpuscles were exceedingly numerous, but in others, with less fever, they occurred in much smaller numbers.

In several cases the fever gradually evinced a more malignant type ending in death. Of two such animals, the one dying on the 39th day after inoculation and the other on the 75th day, blood was drawn previous to death. 10 c.c. of each was injected subcutaneously into a clean horse on April 1st, 1902, which died of typical horse-sickness on April 8th.

During the periods when the disease in these two former horses was assuming a virulent form there were no cases of virulent horse-sickness in the Institute, and it has to be also remembered that the disease is not actively contagious. Even a dose of half an ounce of fluid blood given by the mouth may fail to infect, although a quantity as large as half a pint is generally successful in producing the disease. The incubation period of virulent horse-sickness after subcutaneous inoculation is from 8 to 10 days. The maximum, in my experience during ten years' investigation, has been about 14 days. In the case of the two animals referred to, which died at periods respectively of 39 and 75 days after inoculation, I have already shown that they suffered at frequent intervals from attacks of fever which remitted and intermitted, and the only explanation of their deaths is to admit that the mild and modified fever gradually attained virulence. Such a phenomenon is already well known in malarial fever occurring in man.

I thought proper to denote this form of the disease by the name of the "malarial form of horse-sickness."

Subsequent to this it was suggested that my experiments had been

accidentally contaminated with a disease which is known to occur in horses in South Africa, that is commonly called Biliary Fever.

In answer to such a criticism it has to be stated that no one has yet succeeded in transferring biliary fever from an infected horse to a healthy one in South Africa, in fact that those who have attempted to do so have failed. Under such circumstances it cannot but be surprising that I should succeed again and again in experiments extending over many months, if the condition which I produced had actually been biliary fever.

Biliary fever in the horse is not a disease limited to Africa, indeed it is well known in India. In 1887, Colonel Joshua Nunn, F.R.C.V.S., of the Army Veterinary Department, who had been trained under M. Pasteur, was selected by the Director-General of the Army Veterinary Department, "because of his special fitness for the task, and particularly because of his experience in dealing with destructive horse and cattle diseases in the North-West Provinces of India, where he had gained an excellent reputation," to proceed to S. Africa in order to investigate horse-sickness.

In the valuable report¹, which he made after spending two years in South Africa, he describes the pulmonary form of horse-sickness known as Dunpaardziekte, the Dikkop form, and one which he and also Major Rutherford, A.V.D., describe as the "bilious form of horse-sickness."

It would therefore seem that Colonel Nunn found a disease here which was not, according to his ideas, biliary fever but a variety of horse-sickness.

The results of my post-mortem examinations are in close agreement with the detailed account furnished by Colonel Nunn. I am therefore of opinion that this excellent and careful observer was correct in describing another form of horse-sickness, and it is very probable, while *true* biliary fever exists in this country, that the condition which I have succeeded in producing artificially is neither more nor less than the form of horse-sickness described by Colonel Nunn as the "bilious form."

With a view, however, to meeting criticism by better means than argument, I decided to undertake a critical experiment or series of experiments.

To this end, the stables, which have brick floors cemented on a concrete basis, stone and brick walls, wooden fittings and wooden ceilings, were emptied and kept thus for some weeks. A 1% solution

¹ Report of the Principal Veterinary Surgeon to the Forces to General Viscount Wolseley, Adjutant-General, 27th October, 1888.

of caustic soda was then applied freely to every part including the ceilings with a powerful metal spray. Several days were permitted to elapse, when the whole was again sprayed in the same manner with 1% of formaline. After some days workmen were put in who went over all the brick and stone work filling up any cracks with cement, and all wood-work was also overhauled. Finally the walls and wood-work were repainted.

By the kind assistance of the military authorities, I was now enabled, through the Honourable the Colonial Secretary, to procure ten clean newly imported horses. These were put in trucks at Port Elizabeth, sent to Grahamstown station, from whence they were led directly to the stables. After being placed in the stables, more than a fortnight was permitted to elapse before any experiments were begun. Meanwhile the temperature of each animal was taken five times a day. The most rigorous care was exercised in the case of each animal to exclude any vitiation of the experiment. Each head-stall was thoroughly disinfected; each animal was closely clipped. The food was dry forage which, being cut and stored, was delivered in a special bucket into each manger. Each animal possessed its own water bucket, while the water was taken from that supplied by the town. One horse had to be discarded as being unmanageable.

Examination of the blood.

Each animal, including those used to produce infection, was examined on the average every second day. The want of sufficient staff made it impossible for me to make a daily examination. The blood was drawn from the ear, which was previously shorn and washed with lysol. The cover-glasses were primarily cleaned and kept till required in absolute alcohol. The films were made with strips of papier Joseph and were then fixed with alcohol and ether. Staining was made by the Romanowsky method. Examination was made with Zeiss apochromatic 2 mm. homogeneous immersion objective and compensating oculars 6 and 8.

The examination was made on a Zeiss stand having a mechanical stage fitted with verniers, and the whole cover was systematically examined. Where few parasites occurred more than an hour was required to examine the slide properly. The position on the slide of all parasites found was recorded in my journal and the slides carefully stored. The advantage of this method lies in the fact that the parasites of any case can be found at any time in a few seconds.

EXPERIMENT 1. Two horses, Nos. 27 and 28, were each inoculated on December 4th, 1902, by subcutaneous injection with the blood of "salted" horse *B* which had been reinoculated once 10 days previously with 10 c.c. of virulent blood.

Note. This "salted" horse had not been interfered with since the preceding experiments of the former year. In the former experiments the "salted" horses had been regularly reinoculated each month previous to being used in the experiments.

Horse 27 (Chart I). The temperature rose suddenly eight days after inoculation to 105.8° Fahr. and continued to be high with remissions and some intermissions during thirteen days, after which slighter attacks were noted.

Nine days after inoculation, being the day after the first onset of fever, a few parasites were found in the blood, mostly of the pear-shaped form. On December 15th they were still very few, on the 21st more numerous, while on the 22nd a typical rosette was seen. The term rosette is not quite satisfactory since, as a rule, it consists of four segments arranged as a cross, but in many cases in the form of the imprint of a crow's foot. I think the term "*quadret*" would be more accurate. On the 24th, 26th, 29th and 30th they were again few in number but showed an increase on January 1st. On January 2nd the horse was again inoculated from the same "salted" horse which had not been meanwhile reinoculated. This second inoculation did not produce any severe fever.

On January 4th a "*quadret*" was again seen together with some large more or less circular parasites. Some corpuscles contained two such parasites. On the 6th and 10th they were few in number but showed an increase on January 12th. On the 14th and 17th they were again few and none were seen after this date until February 9th when a quadret was seen. No more were found after this date.

Horse 28 (Chart II). On the evening of December 11th, being seven days after its inoculation, the temperature rose to 101.6° Fahr., reaching 103.8° on the following day, and fever with remissions occurred during the following seven days. The maximum was 107° .

On the day of the first indication of fever no parasites were found, but on January 13th, which coincided with the first remission, a few pear-shaped parasites were seen. They were still few on the 15th, 16th, 17th, and 26th of December, and still nearly always pear-shaped. On December 28th a quadret was seen, and on the 30th the parasites found were nearly all spherical, some being about half the size of a red blood corpuscle.

On January 2nd the horse was reinoculated from the same "salted" horse, but here also no severe fever followed.

A few parasites were subsequently found on January 19th, February 9th, and finally on March 7th, when only one or two were found in a whole slide after a prolonged period.

EXPERIMENT 2. *Horse 29* (Chart III) was inoculated subcutaneously on January 14th with 20 c.c. of the blood of "salted" horse *B*, which had been reinoculated 51 days previously.

Eight days after inoculation the temperature rose suddenly to 104° Fahr. and

fever continued during twelve days, being of the remittent and intermittent type; the maximum being 107.2° .

On the day subsequent to the first occurrence of fever, being January 23rd, a few parasites were found. Three days later a quadret was seen, but also some large spherical forms. On the 28th some spherical forms were seen, while on February 2nd numerous parasites, spherical, pear-shaped, and irregular types, were seen. A few were seen on the 18th, 21st, 24th and 26th of February and on March 5th and April 4th.

EXPERIMENT 3. *To determine whether the blood of an animal suffering from fever, induced by the injection of the blood from a "salted" and reinoculated horse, can induce fever in a clean animal:—*

Horse No. 27 was bled on January 14th and with 20 c.c. of this blood Horse No. 30 (Chart iv) was immediately inoculated by subcutaneous injection.

Eight days after this inoculation the temperature rose to 103.2° . Fever continued with remissions and intermissions during thirteen days, the maximum being 106.8° .

On January 23rd, being the day subsequent to the first elevation of temperature, a few parasites were found. On the 26th they were numerous, after which they disappeared. On February 2nd they appeared in considerable numbers, while coincidentally a very sharp rise of temperature occurred. On February 13th they were still few, as also on the 21st, 24th and 28th days of the same month.

EXPERIMENT 4. *To show*

(1) *That infection remains in a salted horse for at least a year.*

(2) *That if such horse's blood produces infection in a clean horse, this infection protects against further infection with the same salted horse's blood, even when the latter has been reinoculated with virulent blood.*

On February 23rd "salted" horse A, which had not been reinoculated during the past year, was bled. 20 c.c. of this blood was injected into clean Horse No. 31 (Chart v) on the same date. Nine days later the temperature began to rise in the early morning and attained 104.6° , as the highest point, that evening. Thereafter the fever continued with remissions and intermissions during eight days. On March 13th the clean horse was reinoculated with the blood of the same "salted" horse, which had, meanwhile, been reinoculated with virulent blood ten days previously. No rise in the temperature occurred which could be considered to be due to this inoculation.

At later dates the horse was inoculated on two occasions with the blood of goats that had been infected ten days previously with virulent horse-sickness blood.

In this case parasites were found on March 7th, being four days after the first rise of temperature, and were fairly numerous. On the 9th they were fewer in numbers. A few were found on March 23rd and also on the 4th and 14th of April.

EXPERIMENT 5. *To show*

(1) *That the blood of an unsalted horse, although it has been frequently inoculated with salted blood, only conveys a weak infection to a clean horse.*

(2) *That the blood of a goat, infected with horse-sickness blood, can accelerate and intensify that infection.*

A stallion which was inoculated in the former experiments, on several occasions, with salted blood without, however, showing any marked fever, was bled on March 4th. It had not, therefore, received any inoculation during the past year.

Twenty c.c. of its blood were inoculated into clean *Horse* No. 34 (Chart vi) on the same date, March 4th.

During twelve days following no marked rise of temperature occurred, although on the 8th, 9th and 10th days slight rises occurred of which the maximum was 101·8°. No parasites meanwhile were found. On March 16th it was inoculated with 10 c.c. of glycerinated blood derived from a goat which had been inoculated, ten days previously, with 10 c.c. of virulent horse-sickness blood. The temperature rose immediately to 103·2° and a few spherical parasites were found in the blood. During the following day the temperature rose to 106·4° and fell during the night. A second inoculation from a goat with fresh blood was made on the 25th, and on the 27th and 31st of March and 1st of April well-marked rises occurred.

Parasites, which were never very numerous, were seen on the 16th, 18th, 23rd and 27th of March and on the 4th and 14th of April.

EXPERIMENT 6. *To show that the blood of a clean goat, infected ten days previously with virulent horse-sickness, can, even sometimes when glycerinated, convey infection to a clean horse.*

In a previous communication to the Royal Society I showed, when the blood of infected donkeys was preserved, that great differences were found in that taken from different animals. In cattle and goats I have found similar variations in virulence. This fact has to be borne in mind where experiments have to be carried out with blood taken from the donkey, the ox, or the goat.

On February 13th, clean *Horse* No. 32 (Chart vii) was inoculated by subcutaneous injection with 10 c.c. of an equal admixture of blood and glycerine dilution. This dilution consists of equal volumes of water and glycerine containing 1 per 1,000 of pure phenol.

The blood was derived from a goat which was bled ten days after inoculation with 10 c.c. of virulent horse-sickness blood. After mixing the blood with the preservative dilution it was kept at ordinary temperature for two days.

A rise of temperature, slight in degree, occurred on the following day and during six days some fever occurred, the maximum being on the third day, 102·4°.

On the 16th a few parasites were seen, one being a very characteristic "quadret." On the 18th a few were also seen, but from this date onward no more were detected.

EXPERIMENT 7. To show

(1) *That the blood of a clean goat does not convey infection,—*
but (2) *That, after its inoculation with virulent horse-sickness, it does convey infection.*

On March 5th, clean *Horse* No. 35 (Chart viii) was inoculated with 20 c.c. of fresh blood taken from the jugular vein of a clean goat.

During the subsequent twelve days no fever occurred.

Meanwhile this goat had been inoculated with virulent blood and ten days later was bled. The blood was immediately glycerinated and, after 24 hours, 10 c.c. was injected into the same horse. A sharp rise of temperature occurred during the following day, but it is very probable that it was due simply to irritation in the area of the injection. Nine days later the same goat was bled and 10 c.c. of the fresh blood was injected into the horse. Eleven days later the temperature rose slightly, was high the following day and more or less fever persisted during fourteen days.

On April 6th, being the day subsequent to the first real attack of fever, parasites were found in the blood. They were also found on the 7th, 8th, 11th and 15th of the same month.

EXPERIMENT 8. *To show that the blood of a goat infected with virulent horse-sickness fails to infect a clean horse which has already been successfully infected with the blood of a "salted" horse.*

On February 14th, clean Horse No. 33 (Chart IX) was inoculated with 20 c.c. of the blood of "salted" horse B (which had been reinoculated 82 days previously. See also Horse No. 29).

Six days later the temperature began to rise and fairly severe fever persisted, with remissions and intermissions, during the subsequent ten days.

On the 14th and 25th of March and 2nd of April it was inoculated with infected goat's blood without any definite result. The inoculation on March 25th was with the same fresh blood as was used in the preceding experiment.

Parasites were fairly numerous on February 24th, when a "quadret" was also seen. There were but few present on the 26th. A few were also seen on the 2nd and 7th of March.

Summary.

The conclusions I seek to draw from this part of my investigations are:—

1. That a malarial form of horse-sickness probably occurs naturally.
2. That when animals which are either naturally relatively susceptible, such as the donkey, ox, and goat, or animals which have acquired protection, *e.g.* "salted" horses, are inoculated with virulent horse-sickness blood, their blood conveys a modified infection of horse-sickness which is malarial in type.
3. That this malarial form is associated with the presence of parasites within the red corpuscles of the blood of the infected animal.
4. That the blood of "salted" horses which have been previously regularly inoculated during several months is of a dangerous order of virulence. *Vide* former experiments.

20a

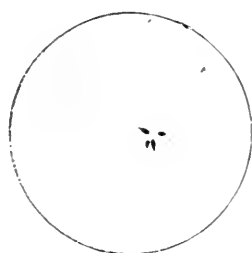


Fig. 1.



Fig. 2.

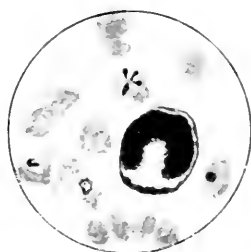


Fig. 3.



Fig. 4.

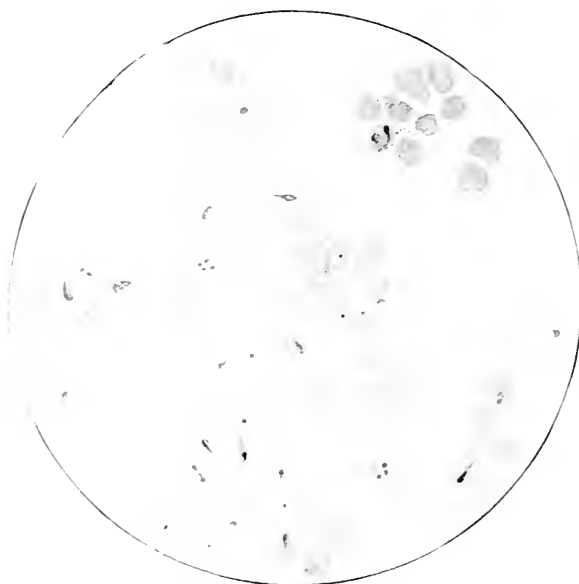


Fig. 5.

5. That the blood of "salted" horses which have only been once inoculated after several months' rest conveys a fairly severe fever.

6. That the blood of "salted" horses, which have not been inoculated for even a year, can still convey infection.

7. That "unsalted" horses which have been inoculated a year previously with "salted" blood convey only a weak infection.

8. That a severe attack of malarial horse-sickness gives a considerable degree of protection against subsequent inoculation with "salted" blood.

9. That when regularly inoculated "salted" horses are used to produce blood giving severe fever, such fever may become so severe as to cause death with symptoms of horse-sickness.

10. That the blood of the latter animals which die can produce virulent horse-sickness in clean horses, if used in a considerable dose, *i.e.* 20 c.c.

DESCRIPTION OF PLATE I.

Fig. 1. A field from the blood of *Horse* No. 32. The careful examination of a whole coverglass only revealed two parasites, of which one is seen in the plate. Subsequent to this the febrile condition came to an end and the parasites totally disappeared.

Fig. 2. A field from the blood of *Horse* No. 27. Only a very few parasites were found at this date, and in this case also the appearance of a "quadret" with very few parasites of other form was followed by the disappearance of fever and parasites during a considerable time.

Figs. 3 and 4. Fields from the blood of *Horse* No. 29 seen on the same day. In this case a "quadret" is also seen but also many other forms, and this condition was followed by a sharp attack of fever and the appearance of numerous parasites.

The above cases were all mild in type.

Fig. 5. A field from the blood of *Horse* No. 16 of the former experiments. This case was extremely severe in type, and the animal died on the day following that on which this field was observed.

Note. Photographs of water-colour figures. In Figs. 1 and 5 the corpuscles are pink, the bodies dark-blue. In Figs. 2, 3, and 4 the corpuscles are violet, the bodies dark-blue or (Fig. 4) violet-blue. The figures show no indication of chromatin staining.—*Ed.*

CHART I. To show that the blood of a salted horse, which had been reinoculated 10 days previously with virulent horse-sickness, can set up infection in a clean horse.

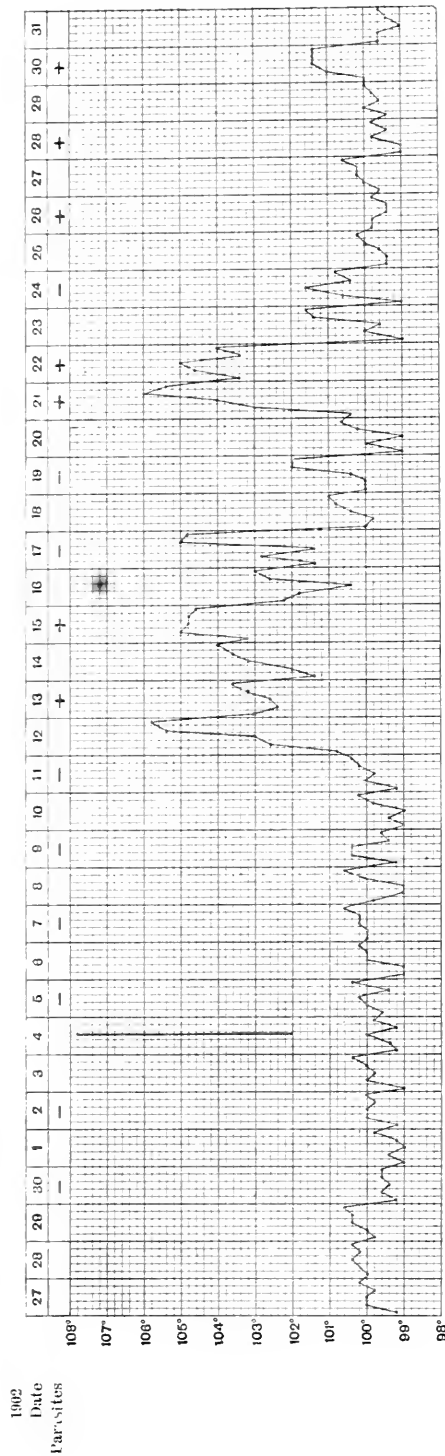
Disease, Horse-Sickness.

Horse No. 27.

Infecting Material, 20 c.c. subcutaneously of blood of salted horse B 4, XII, 02 (inoculated 10 days previously with 10 c.c. preserved virulent blood).

Nov.

Dec.



Jan.

Feb.

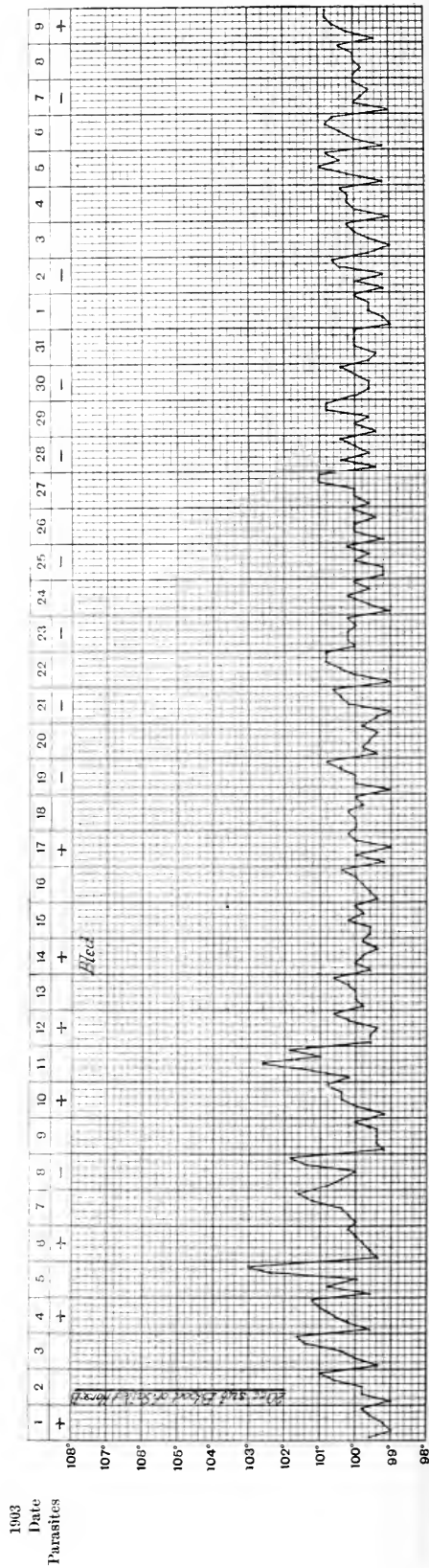


CHART II. To show the condition as in preceding No. 27.

Disease. Horse-Sickness. Horse No. 28.

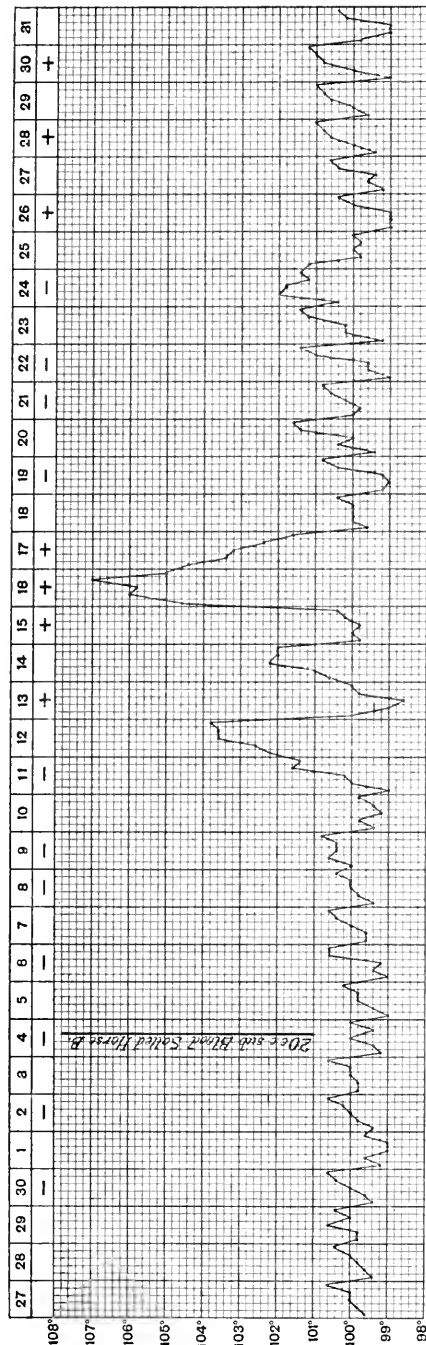
Injecting Material. 20 c.c. blood of salted horse B injected subcutaneously on 4. XII. 02 (Horse B was inoculated ten days previously with 10 c.c. virulent blood).

Nov. Dec.

1902

Date

Parasites



Jan.

1903

Date

Parasites

Feb.

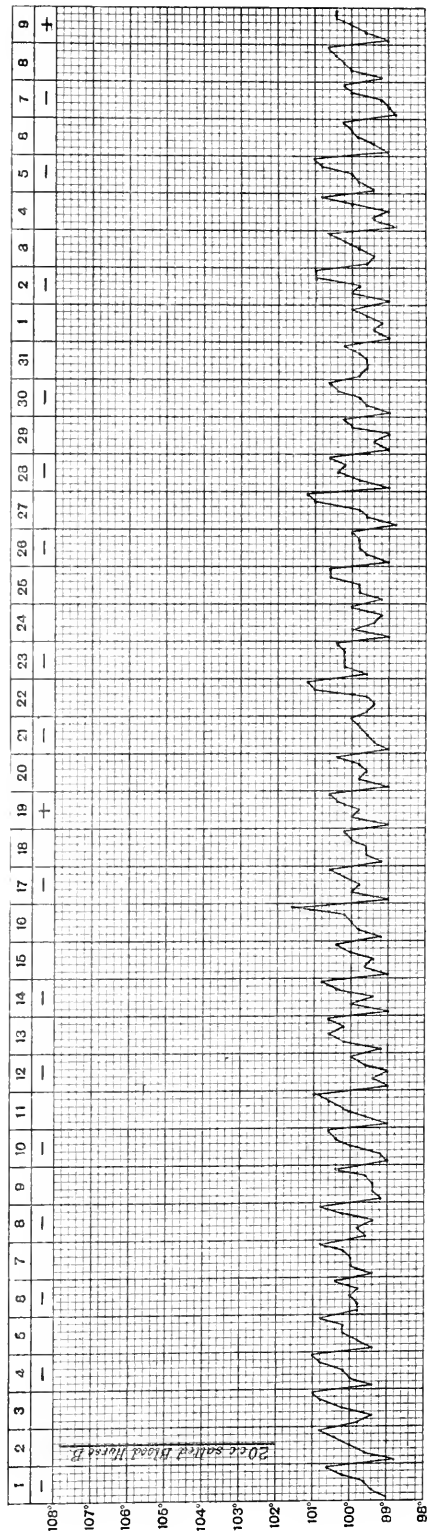
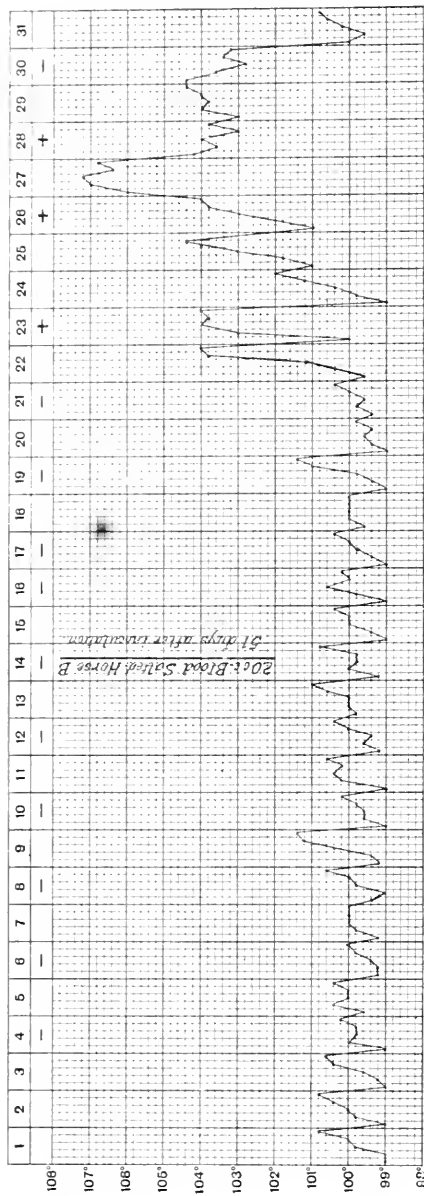


CHART III. To show that the blood of a salted horse, which had been reinoculated 51 days previously with virulent horse-sickness, can set up infection in a clean horse.

Disease. Horse-Sickness. Horse No. 29.

Infecting Material. 20 c.c. subcutaneously blood of salted horse B.
Jan.

1903
Date
Parasites



Feb.

1903
Date
Parasites

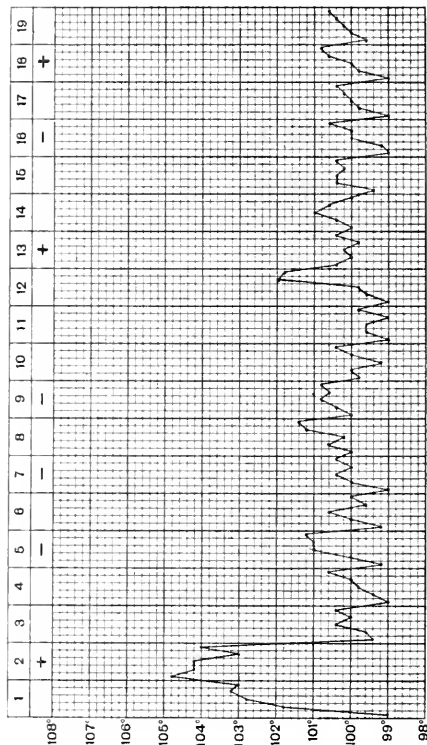


CHART IV. To show that the blood of a horse, suffering from malarial horse-sickness, can induce the same fever in a clean horse.

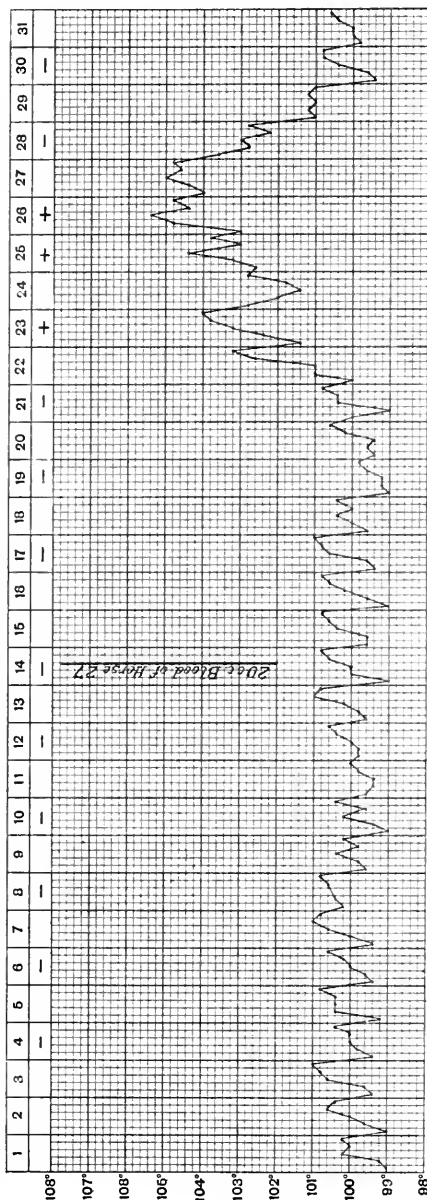
Disease. **Horse-Sickness.** Horse No. 30. Infecting Material. 20 c.c. subcutaneously of the blood of Horse 27 which was suffering from fever induced by injection with "salted" blood.

Jan.

1903

Date

Parasites



Feb.

1903

Date

Parasites

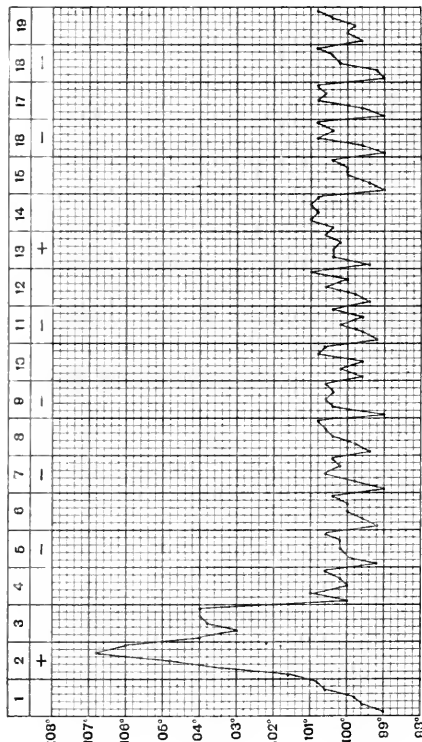
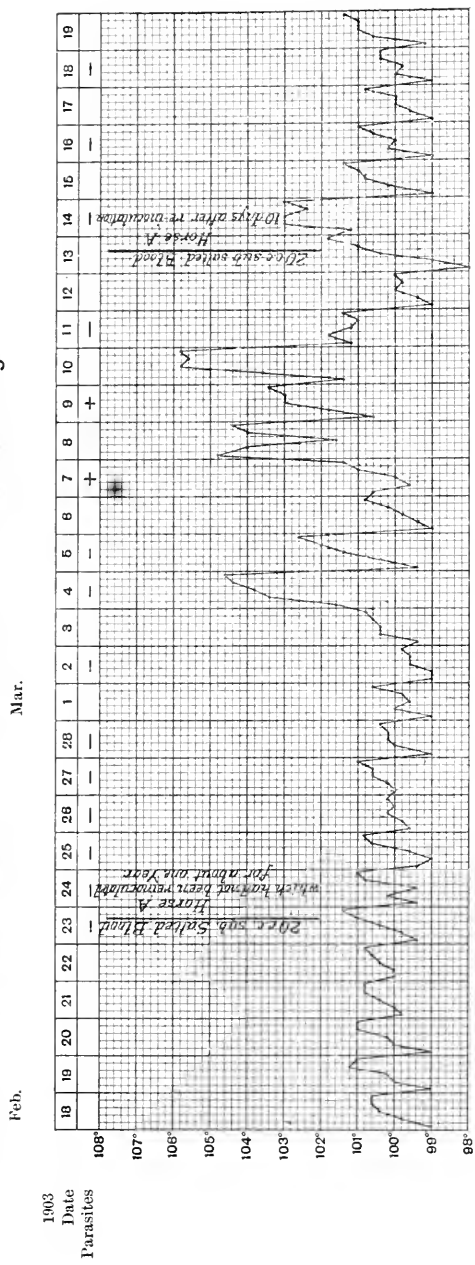


CHART V. To show: (1) that infection remains in a "salted" horse for at least a year; (2) that if such a horse's blood produces infection in a clean horse, this infection protects against further infection with the same salted horse's blood, even when the latter has been reinoculated with virulent blood.

Disease. Horse-Sickness. Horse No. 31.



April

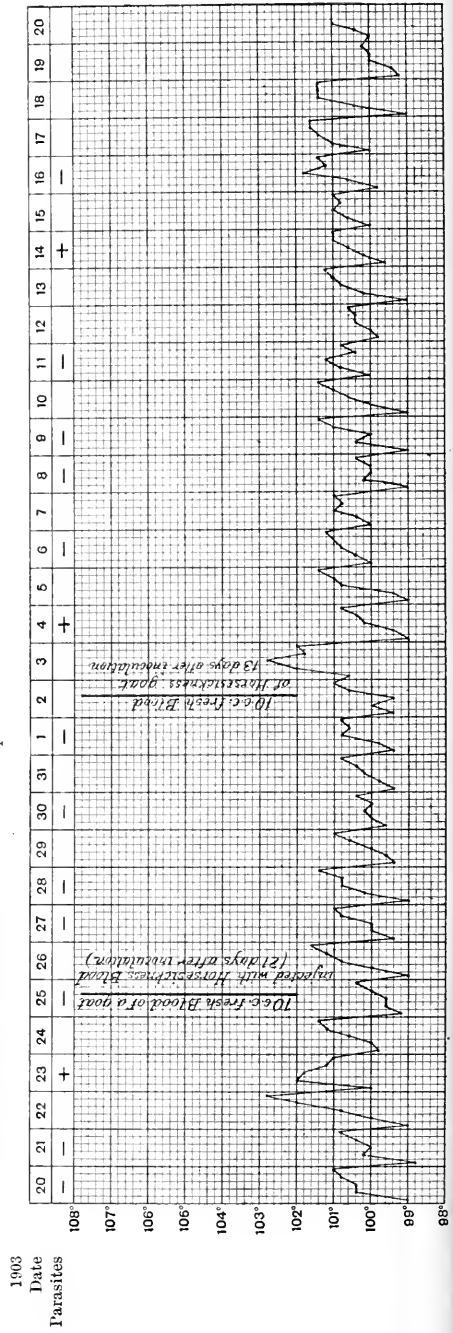
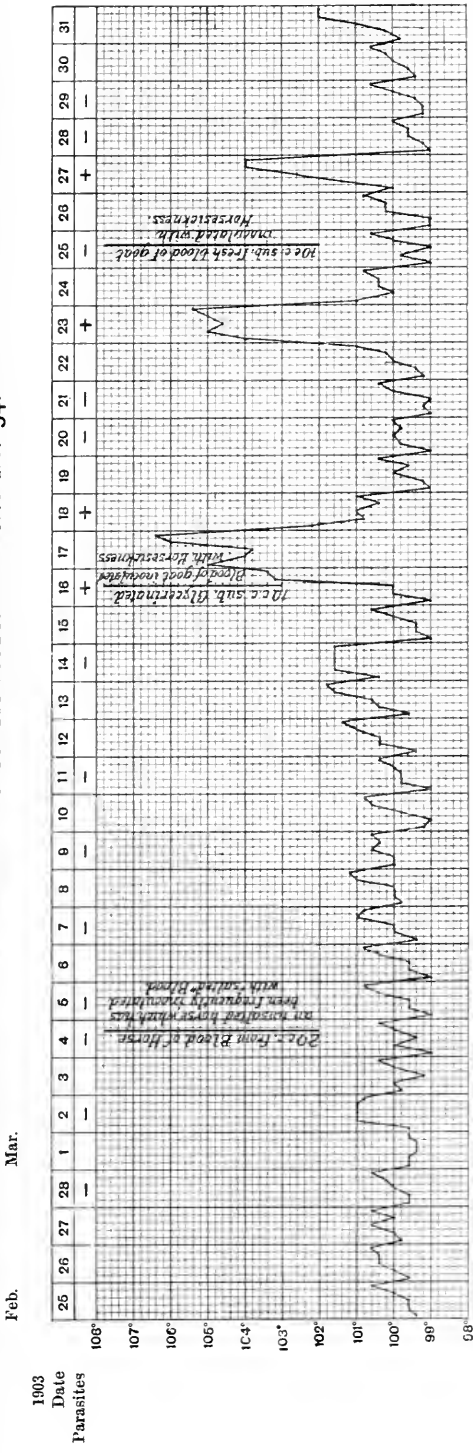


CHART VI. To show that: (1) the blood of an unsalted horse, although it has been frequently inoculated with salted blood, only conveys a weak infection to a clean horse; (2) the blood of a goat, infected with horse-sickness, can accelerate and intensify that infection.

Disease. Horse-Sickness. Horse No. 34.



April

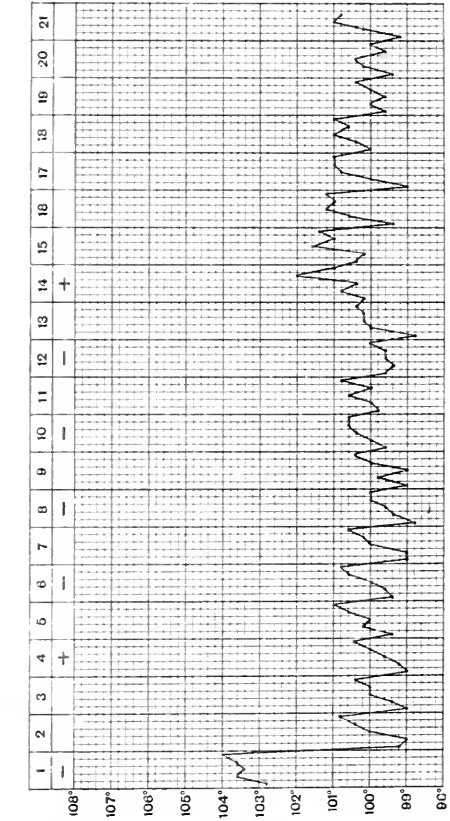


CHART VII. To show that the blood of a clean goat, infected with virulent horse-sickness, can convey the malarial form to a clean horse.

Disease. Horse-Sickness. Horse No. 32.

Mar.

Feb.

1903
Date
Parasites

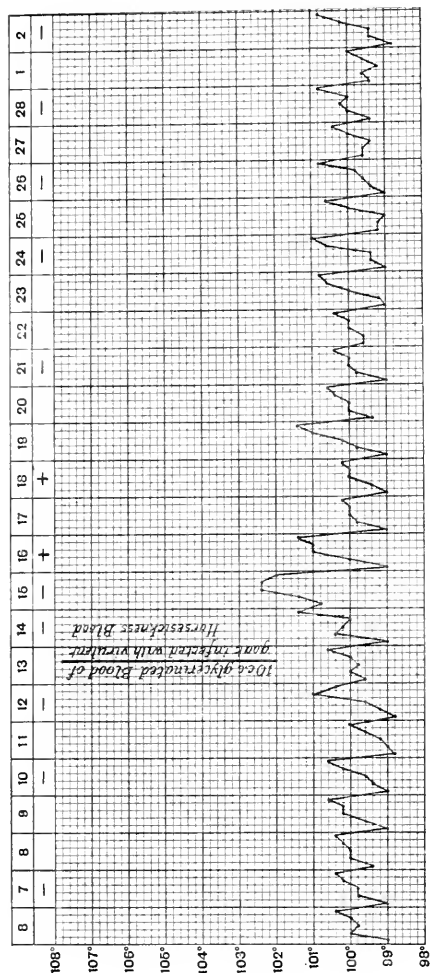
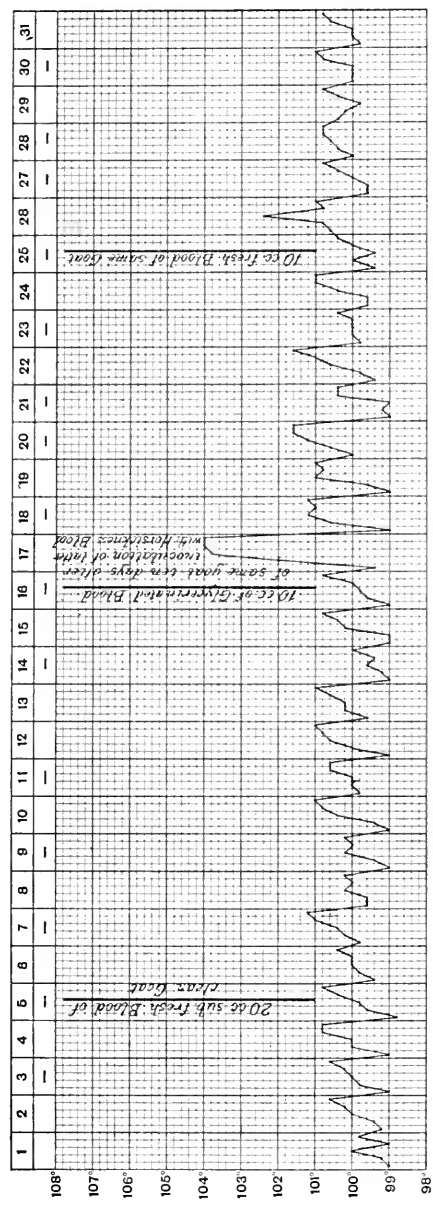


CHART VIII. To show: (1) that the blood of a clean goat does not convey infection, but
 (2) that after its inoculation with virulent horse-sickness, it does convey infection.

Disease. **Horse-Sickness.** Horse No. 35.

Mar.

1903
 Date
 Parasites



April

1903
 Date
 Parasites

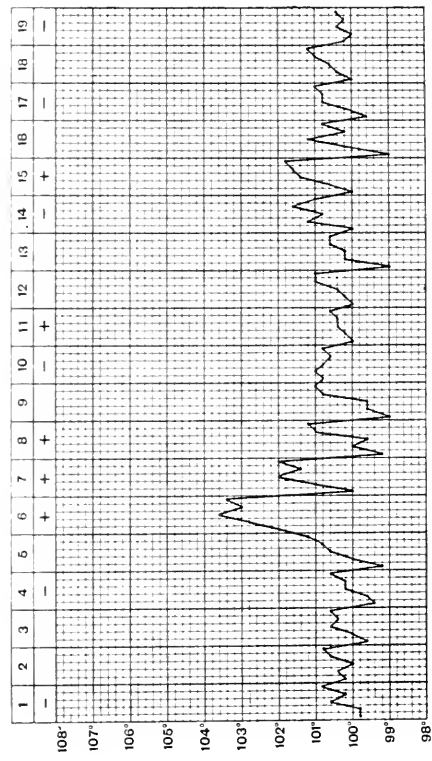
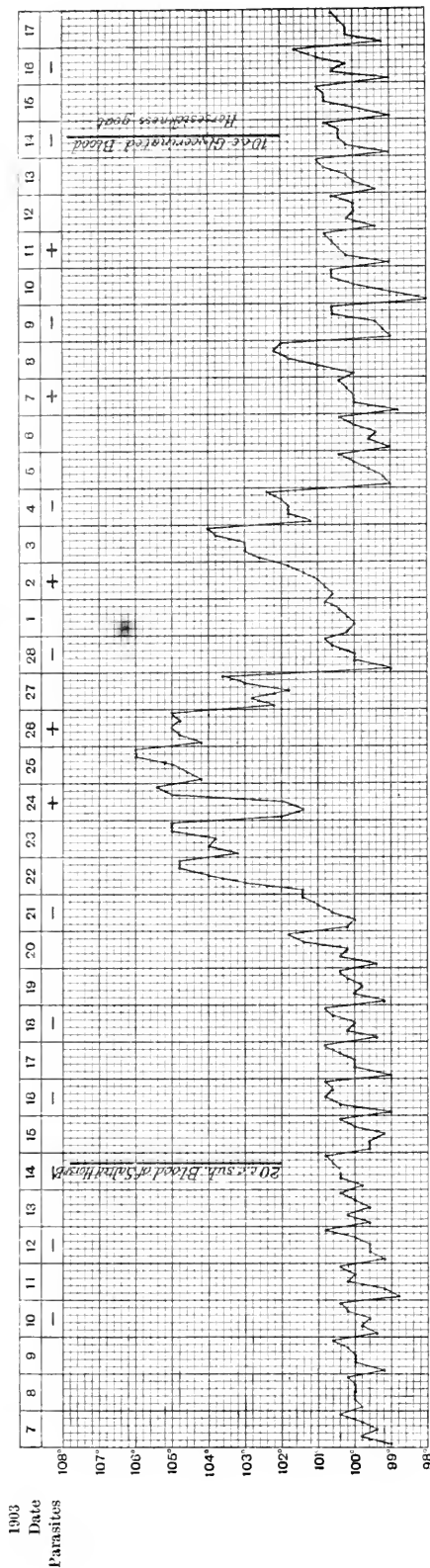


CHART IX. To prove that the blood of a goat infected with virulent horse-sickness fails to infect a clean horse,
which has already been successfully infected with the blood of a "salted" horse.

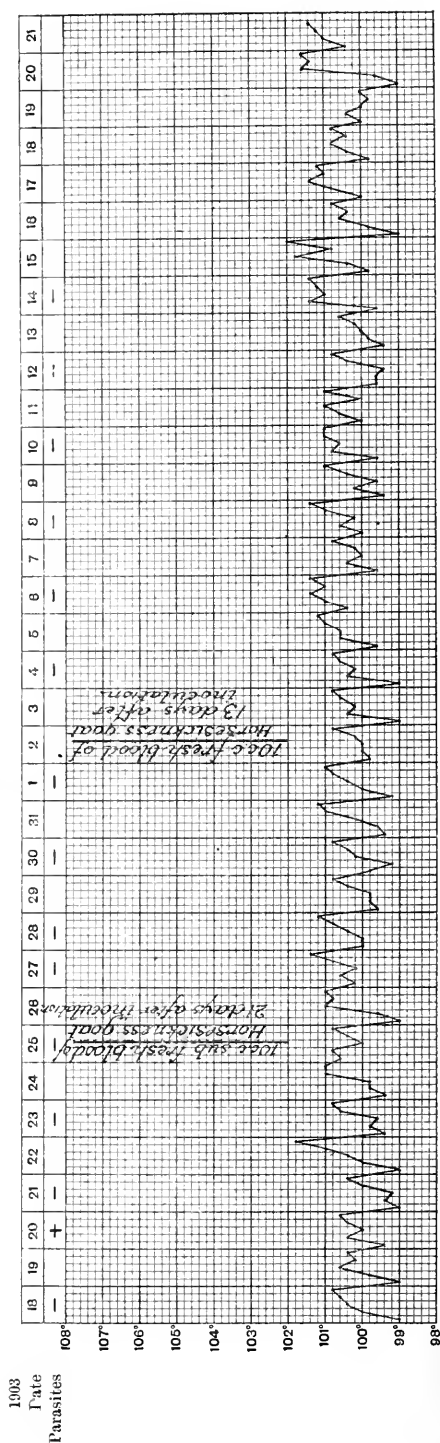
Disease. **Horse-Sickness.** Horse No. 33.

Feb.

Mar.



April



SOME FUNDAMENTAL EXPERIMENTS ON IMMUNITY, ILLUSTRATED.

By E. F. BASHFORD, M.D.,

*General Superintendent and Director of Central Laboratory,
Cancer Research Fund, Grocers' Scholar, &c.*

*Repetition of Fundamental Experiments on some Phenomena
accompanying Artificially Produced Immunity, as observed when one
Species of Animal has been Immunised against the Erythrocytes of
another Species.*

It has been my experience that written descriptions of experiments on immunity cannot convey to the reader anything like so forcible an impression as a demonstration of the actual experiments themselves. On account of the long preparation necessary for the demonstration of what are but rapidly passing phenomena an opportunity of viewing them presents itself to very few. I have sought for some method of demonstrating to a wider public than the select few, having opportunity of performing similar experiments, the real value of some investigations in this field of enquiry. Since Ehrlich substituted experiments in the test-tube for experiments on living animals the advance in our knowledge has been great, so great, indeed, that we have hardly had time to properly correlate the new facts which have accumulated, and still less opportunity to impartially appreciate the value of the hypotheses which have been formulated for their explanation. If this paper contribute a little in assisting others to separate the facts from the hypotheses, and to recognise that the explanatory hypotheses and technical phraseology which form so large a part of the literature on immunity have a definite relation to undoubted facts, it will, I hope, fulfil a useful purpose. I have repeated the fundamental experiments of others, and especially those of Bordet and of Ehrlich and Morgenroth,

and I have utilised the actual experiments themselves to obtain graphic records.

The facts related in this paper are for the most part well-known, and they are not exhaustively discussed, but they are presented in a form which differs from that which is customary. The plates seem to me to be of chief importance, and to them an explanatory description is merely appended¹.

My avoidance of the terminology employed by Bordet, Ehrlich and Morgenroth, is solely due to my desire to escape the necessity of using terms, which, although applied to definite phenomena, are so applied as to involve hypothetical conceptions of their significance. I wish to fully acknowledge my indebtedness to the work of those distinguished investigators, and to the work of Max Neisser, v. Dungern, and Hans Sachs.

Methods of Experiment.

My experiments were performed *in vitro*, chiefly because it was possible to satisfy oneself that the phenomena thus observed corresponded to similar phenomena *in vivo*. For *in vitro* experiments of this nature it is essential that all test-tubes, pipettes, vessels, and saline used be sterile. The saline was always 0.86 % solution. Bullocks' blood was used to induce the immunity in rabbits². For special purposes experiments were made with guinea-pigs.

In all cases, except where otherwise stated, serum was obtained by bleeding the rabbits from the marginal vein of the ear. The blood was received in a sterile porcelain capsule and *gently* defibrinated with a sterilised piece of wood. The blood was then well centrifugalised. By this method serum is more quickly obtained and in greater quantity than by allowing it to separate by coagulation of the blood. One can easily and repeatedly obtain 20 c.c. of blood from the ear vein of a rabbit weighing about 2 kilos. With careful defibrination there is rarely excess of blood

¹ The following studies formed a portion of the preliminary stages in an extensive investigation into the mechanism of antitoxine production. New duties have interfered with the satisfactory completion of this work. The subject-matter of this paper formed part of an introduction to a progress report submitted to the Scientific Assessors of the Worshipful Company of Grocers, April, 1902. For a critical discussion of the theoretical explanations of some of the facts related in this paper I would refer to the recent papers of Professor R. Muir (*Lancet*, August 15th, 1903), and Dr Ritchie (*Journal of Hygiene*, 1902), and to the literature there quoted.

² It is scarcely necessary to point out that immunity to blood implies immunity to all the different constituents of blood. Only some of the manifestations of immunity to the erythrocytes are considered in this paper.

pigment in the serum. It was thus also possible to utilise when necessary both corpuscles and serum. The bullocks' blood was obtained from the abattoir quite fresh and sterile for each determination. Every estimation of a reaction, and whatever was dependent on it, was carried through to a finish without interval. This was done to obtain regularity in the results and to avoid fallacies attributable either to any spontaneous weakening of action due to sera having been kept for many hours, or to increased susceptibility of the bullock's corpuscles, owing to the same cause. This made the work often very arduous, but this was necessary where great stress was being laid on the comparison of degrees of actions manifested.

In such a series of experiments each test-tube contains the same quantity of blood suspension. The serum to be tested is added in decreasing quantities to the constant quantity of blood in each tube of the series. This necessitates the employment of the serum in convenient dilutions and diminishing quantities of each dilution. Therefore to the test-tubes receiving the lesser quantities of serum, or diluted serum, amounts of normal saline must be added to equalise the volume of fluid in the tubes throughout the series.

When any phenomenon is referred to as being manifested on blood, it is to be understood, that in all cases 1 c.c. of a 5 % suspension of fresh bullock's blood in 0.86 % saline is meant. The serum had not been removed from the bullock's blood.

Throughout the experiments the dilutions of the sera of which the action was being determined were 1 : 1, 1 : 10, 1 : 100, 1 : 1000, 1 : 10,000, 1 : 100,000, etc. When in a long series of test-tubes the quantities of serum added to the standard quantity of bullock's blood were 1.0 c.c., 0.75 c.c., 0.5 c.c., 0.35 c.c., 0.25 c.c., 0.15 c.c. of each dilution respectively, a comparable series was obtained with regularly diminishing quantities of the active agent. The quantities of saline added were of course 0.0, 0.25, 0.5 ; 0.65, 0.75 and 0.85 respectively. The test-tubes were conveniently arranged in stands holding three rows of six each ; one row for each dilution. The results in the corresponding tubes in different rows (which all had the same quantity of fluid, but different dilutions of the active agent, added to them) represented the effect of the tenth or the hundred-fold multiples of the agent added. Some such arrangement was required in order to facilitate the manifold measurements of fluid sometimes necessary, to allow of uniformity in time occupied, and of compactness in each experiment. In making colour-index comparisons, for example, those given in the graphic records which follow, it is essential that care be taken to add saline in the quantities necessary to raise the amount of fluid in each test-tube to the same quantity. So soon as the necessary mixture had been prepared all the tubes in the series were well shaken, and then placed along with the necessary control tubes for two hours at 37—38° C., after which the result was noted. The tubes were then left in the cold for 12 to 15 hours, and the results again controlled. The duration of stay in the incubator and the degree of shaking to which the tubes have been subjected are important factors in limiting the actions manifested. In experiments such as those about to be described it is important to pay as much attention to the limit where action is no longer visible, as to the limit where complete action manifests itself. The former is a very necessary control, in any single series of experiments, and in comparing the results obtained in series of experiments of different dates.

I. *Relative Indifference of Rabbit's Serum to Bullock's Erythrocytes.*

Normal rabbit serum is an almost indifferent medium for bullock's erythrocytes when the latter are suspended therein. The following experiment illustrates this statement. Each of a series of six test-tubes received 1 c.c. of blood suspension followed by varying quantities of normal rabbit's serum. Saline was added in sufficient quantity to raise the amount of fluid in each test-tube to 2 c.c. All the tubes were then shaken, and placed at rest for two hours in the incubator. At the expiration of this period the corpuscles settle down to a considerable extent and the practised eye can detect and judge if there is evidence of laking (haemolysis) of the corpuscles. This for guidance in the manipulations which follow. The tubes were then placed at 11–12° C. for 12 hours and the result recorded.

Usual Method of Recording such Experiments.

A series of test-tubes receive 1 c.c. of 5 % blood suspension, varying amounts of normal rabbit's serum, and saline q.s. to raise the total volume to 2.0 c.c. in each tube. Such an experiment is usually recorded as follows:

5 % suspension of Bullock's blood in c.c.	Amount of normal rabbit's serum in c.c.	Amount of saline added in c.c.	Result
1.0	1.0	0.	Trace of Hb diffused through lower layers of fluid
„	0.75	0.25	Minute trace of Hb diffused through lower layers of fluid
„	0.5	0.5	Nothing
„	0.35	0.65	„
„	0.25	0.75	„
„	0.15	0.85	„
„	Control + 1 c.c. saline, result = nothing		

The above method of recording the experiments conveys no idea of the appearances to one unfamiliar with them, and does not yield very objective data for comparison with future experiments. Before alluding to what appears to me to be a better method it will be well to consider an experiment with the serum of an immune animal.

II. *Production of Immunity to Erythrocytes*¹.

If 20 c.c. of fresh bullock's blood be injected into the peritoneum of a rabbit, after an interval of a few days (8 days in the following experiment), its serum will be found to have ceased to be a relatively indifferent medium when bullock's corpuscles are suspended in it. Under the previously described experimental conditions bullock's corpuscles now undergo complete laking. To the blood there was added serum undiluted, diluted 1 : 10 and 1 : 100. The experiment is usually recorded as follows :

5% suspension of Bullock's blood in c.c.	Amount of immunised rabbit's serum in c.c.	Amount of saline added in c.c.	Result	
1·0	1	0·0	Complete laking	
”	Undiluted	0·75	0·25	” ”
”		0·5	0·5	” ”
”		0·35	0·35	” ”
”		0·25	0·75	? ” ”
”		0·15	0·85	” ”
”	Diluted	0·1	0·0	” ”
”		0·075	0·25	” ”
”		0·05	0·5	” ”
”		0·035	0·65	” ”
”		0·025	0·75	” ”
”	Diluted	0·015	0·85	” ”
”		0·01	0·0	Trace of laking
”		0·0075	0·35	? ” ”
”		0·005	0·5	Nothing
”		0·0035	0·65	”
”	1 : 100	0·0025	0·75	”
”		0·0015	0·85	”
”	Control + 1 c.c. saline = nothing			

For a graphic record of the two preceding experiments see Plates II and III.

Graphic Method of recording the Results.

In order to obtain records of a large number of experiments on immunity I have employed a method which occurred to me seven years ago when engaged in some clinical observations with my respected teacher and friend, Sir Thomas R. Fraser. At that time we found it convenient to roughly record the amount of bile-pigment present in the

¹ See footnote No. 2 on p. 32.

serum in cases of jaundice, and the amount of haemoglobin free at different dates in the serum in a case of paroxysmal haemoglobinuria. We did this by placing drops of serum upon white blotting-paper. The records then made remain comparable at the present time. I have utilised this method to obtain, within small compass, compact records of several series of related experiments, and to obtain details of each test-tube experiment in a series.

In the one case (Method I), drops from the clear fluid from above the corpuscular sediment were placed in a series upon glazed paper. In the other case (Method II), immediately after shaking each test-tube, equal quantities of the fluid (and also therefore of suspended erythrocytes, if any were present) were transferred to white blotting-paper and allowed to diffuse. When dry both forms of record were permanent.

The technique of Method I is as follows. The tubes are gently agitated to mix the supernatant fluid as much as possible without disturbing the corpuscular sediment. Measured quantities of the fluid are now withdrawn from each test-tube by means of a marked capillary tube, and expelled in series upon the surface of a sheet of glazed paper. The drops dry on the paper and at once yield a graphic and accurate record of the actual experiment. (See Plate III, p. 68.)

Method II. Since the total volume of fluid in each of a series of test-tubes is the same, equal fractions thereof will yield comparable records. From each test-tube, immediately after shaking, a measured quantity of fluid is withdrawn by means of a fine pipette. The fluid is allowed to gradually diffuse from the tip of the pipette gently resting on white blotting-paper. By this procedure one obtains a series of circular diffusion areas which register the phenomena observed in the corresponding test-tube from which they were derived. In the test-tubes at one end of the series the erythrocytes were unaffected, they settled to the bottom, leaving a clear watery zone of fluid above. On the contrary, if haemolysis occurs, the amount of sediment is diminished, and the fluid is tinged with haemoglobin in accordance with the degree of the solution of the erythrocytes. Where complete laking takes place toward the end of the series there is no sediment, and the fluid is uniformly tinged with haemoglobin. The limits of diffusion of fluid and erythrocytes on blotting-paper do not coincide. The erythrocytes, when present in a diffusing fluid, never spread as far as the fluid, but remain localised about the centre whence diffusion has taken place. The two limits reached produce a circular zone on the blotting-paper. This circular zone is most marked where erythrocytes have not parted with

any of their haemoglobin. The zone is then colourless, and its outer limit ill-defined. On the contrary the zone is coloured if the fluid has contained free haemoglobin, and at the same time the margin reached by the fluid becomes more distinct owing to the accumulation of the blood pigment there. The marginal zone becomes less definite as the amount of free haemoglobin increases and the number of suspended erythrocytes diminishes. It disappears altogether when there has been complete haemolysis, for then a diffusion of the haemoglobin uniform with that of the fluid is possible, although a deeper shade is produced at the margin. The erythrocytes naturally diffuse uniformly, but if they have been clumped together their diffusion is proportionately irregular.

On any radius of such a circular diffusion area one has a reproduction of the appearances presented in the corresponding vertical test-tube. The erythrocytes which would have been deposited in the test-tube are represented by those occupying the central area in the diffusion on blotting-paper, and the zone of fluid in the test-tube is reproduced in the marginal zone of the diffusion area. The marginal zone on the blotting-paper also shows the character of the fluid in the test-tubes, and with the disappearance of the distinction between fluid and sediment in the test-tube the distinction between marginal zone and central area is lost in the uniformity of the appearance of the diffusion area (see Plates II and VIII).

By utilising graphic records obtained by the methods described, all the phenomena referred to in this paper have been illustrated. As regards the occurrence of these phenomena there is no room for doubt, although great diversity of opinion exists with regard to their hypothetical explanation.

III. *Experimental Modifications of the Properties of Immune Serum.*

(1) *Deprivation of acquired character.* (Effect of heat.) An immune serum differs from a normal serum in that it has acquired the power to produce laking of bullock's blood (see Experiment II). When heated in a water-bath at 56° C. for half-an-hour it becomes an even more indifferent medium than the serum of an untreated rabbit:

	1.0	c.c.	devoid of evident action
Heated	0.75	„	„
Immune	0.5	„	„
Serum	0.35	„	„
Undiluted	0.25	„	„
	0.15	„	„

Experiments on Immunity

	1.0	c.c. devoid of evident action	
Diluted 1 : 10	0.075	"	"
	0.05	"	"
	0.035	"	"
	0.025	"	"
	0.015	"	"
Diluted 1 : 100	0.01	"	"
	0.0075	"	"
	0.005	"	"
	0.0035	"	"
	0.0025	"	"
Control + 1	0.0015	"	"
	c.c. Saline	"	"

This corresponds to Experiment III on Plate III, and Experiment V on Plate II.

(2) *Restoration of acquired character.* Heated immune serum is placed in a series of test-tubes as above and normal rabbit serum is added thereto, in a quantity (represented by test No. 6, in Experiment I on Plate III), which of itself is devoid of all evident action. The result is as follows :

	c.c.	
Heated immune serum + fresh serum undiluted	1.0	Hb completely diffused into the fluid
	0.75	
	0.5	
	0.35	
	0.25	
	0.15	
Diluted 1 : 10	0.1	Diffusion of Hb diminishing
	0.075	
	0.05	
	0.035	
	0.025	
Diluted 1 : 100	0.015	Diffusion of Hb diminishing
	0.01	
	0.0075	
	0.005	
	0.0035	
Diluted 1 : 1000	0.0025	? Trace of Hb diffused out Nothing
	0.0015	
	0.0001	
	0.00075	
	0.0005	
Control + 1	0.00035	"
	0.00025	"
	0.00015	"

Control + 1 c.c. Saline = Nothing.

This experiment corresponds to No. IV on Plate III.

(3) *Augmentation of the acquired character.* The last experiment shows not only that heated immune serum can have its lost power of laking restored, but that the power of laking may be greatly augmented.

If to the same dilutions of the immune serum used in Experiment II (*i.e.*, unheated serum) there be added normal rabbit's serum as in the last experiment, a similar and slightly greater augmentation of the laking power is manifested. This is due to the amount of the factor which is normally present in the immune serum not having been eliminated by previous heating.

	c.c.	
Serum Undiluted	1.0	} Complete laking
	0.75	
	0.5	
	0.35	
	0.25	
	0.15	
Diluted 1 : 10	0.1	} Complete laking
	0.075	
	0.05	
	0.035	
	0.025	
	0.015	
Diluted 1 : 100	0.01	? Complete laking
	0.0075	} Amount of laking diminishing
	0.005	
	0.0035	
	0.0025	
	0.0015	
Diluted 1 : 1000	0.001	} Trace of laking
	0.00075	
	0.0005	? „ „
	0.00035	Nothing
	0.00025	„
	0.00015	„
Control + 1 c.c. of Saline = Nothing.		

This corresponds to Experiment V on Plate III.

Difference between Normal Serum and that of an Immunised Animal.

The power of laking bullock's corpuscles has been acquired by what is practically a process of immunisation. As a concomitant consequence the serum of the rabbit has become endowed with a power previously absent. This power is specific. The acquirement of this power of itself distinguishes immune serum from the serum of a normal rabbit. The following experiment demonstrates that immune rabbit serum exerts no effect upon the erythrocytes of a normal rabbit. In this case normal rabbit's erythrocytes were freed from their own serum by centrifugalising and washing four times as a 5% suspension in 0.86% saline.

The rabbit's erythrocytes (1 c.c. of 5% suspension) were then subjected to the influence of the immune serum.

Amount of immune
rabbit's serum in c.c.

1.0

In all the tubes the corpuscles settled down,
leaving the fluid above them clear.

0.75

0.5

0.35

0.25

0.15

Control + 1 c.c. Saline = Nothing.

Control for colour, Serum 0.25 + 0.25 Saline.

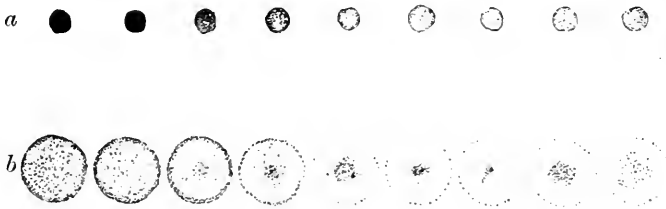
This corresponds to Experiment VI on Plate III, and illustrates the general law that the serum of the immunised animal remains innocuous for the cells of its own species. Exceptions to the specific nature of this phenomenon have indeed been reported, but as this question is still the subject of controversy I refrain from further discussion of the topic.

IV. Agglutinating Power also acquired.

There is often (as shown in the accompanying figure) a somewhat abrupt disappearance of the phenomena of laking, so that the range of haemolytic action does not descend so far in the series of test-tubes as would be expected.

If the tubes in which the expected haemolysis has not shown itself be shaken, the bullock's corpuscles will be found clumped

together, an occurrence which personally I have never observed to result from the action of normal rabbit's serum. The following experi-



To show how the occurrence of agglutination interferes with the lower limit of haemolysis.

a. Fluid only on glazed paper. *b.* Fluid with suspended corpuscles, if present, diffused on blotting-paper.

ment illustrates well what is meant. In the dilution of 1 : 100 a serum was still able to induce complete laking. The laking was almost complete with 0·005 c.c., but was unexpectedly almost absent with 0·0035 c.c. as shown below. The tubes in the series containing higher dilutions are not quoted.

	Amount in c.c.	Result
Immune serum 1 : 100	(1) 0·01	Complete laking
	(2) 0·0075	„ „
	(3) 0·005	Trace of „
	(4) 0·0035	Trace of laking, almost complete agglutination
	(5) 0·0025	? Trace of laking, almost complete agglutination
	(6) 0·0015	No trace of laking, almost complete agglutination
	(7) 0·001	Agglutination diminishing
	(8) 0·00075	
	(9) 0·0005	Trace of agglutination
	(10) 0·00035	Nothing
	(11) 0·00025	„
	(12) 0·00015	„

Control + 1 c.c. Saline = Nothing.

The phenomena exhibited in such a series are very well shown in Experiment II on Plate II.

Laking and Clumping independent phenomena.

The phenomenon of agglutination is quite independent to that of haemolysis to which, at one time, it was regarded as the necessary preliminary. By suitable experiments the entire independence of the phenomena can be demonstrated. For example, a degree of heating in

the water-bath which will abolish the power of laking will leave unimpaired the power to agglutinate. This loss of power to lake and retention of the power to agglutinate is demonstrated in Experiment III, on Plate II. On the same record a further demonstration of the independence of the two phenomena is given, for an acquired power to lake is present in the absence of agglutination. The power to lake may be abolished by heating. In the above case the heating was for $\frac{1}{4}$ hour at 56°C ., but for any particular serum the degree of temperature and duration of heating has to be worked out in order to ascertain the best experimental conditions. At a definite temperature and with sufficiently prolonged exposure both the power to agglutinate and to lake are permanently abolished.

The agglutination of the red blood corpuscles is believed to be analogous to the clumping of bacteria by specific immune sera. The laking of the corpuscles or haemolysis is held to be similar to bacteriolysis.

The Serum of an Immunised Animal has acquired New Properties which do not cause, but merely accompany Immunity.

From the six fundamental experiments demonstrated on Plate III, the conclusion has been drawn that the serum of a rabbit which has been immunised against the blood of the bullock has had two new properties superadded to those naturally present. In addition to a something which is very susceptible to heat and normally contained in rabbit's serum, other bodies are present which owe their origin to the injection of the bullock's corpuscles, and which for the sake of clearness we will simply regard as concomitants of immunity. The acquired powers to agglutinate and to lake are thereby explained.

Much confusion has been introduced into these matters by the fact that the something present normally in rabbit's serum, and also in the serum of the immunised rabbit, is enabled to effect more ready liberation of the haemoglobin contents of the erythrocytes if these have been acted upon by the serum of an immunised animal. Extensive investigation has shown that the diffusion of haemoglobin is but an incidental phenomenon in the reactions here taking place. Some believe that the erythrocytes are destroyed by the actions of osmosis in a manner exactly analogous to the action of distilled water on them. Others assert that osmotic laws play only a minor part, and that the red cells are dissolved in consequence of a chemical union between them, the products due to immunity, and something normally present in serum. The reaction requires to be analysed in order that the relationship of the different factors may be made more evident.

V. *Analysis of the Mechanism by which Erythrocytes are laked.*

(1) *Preliminary determination of the three constants necessary for complete haemolysis.* The following experiments were performed when an opportunity of using a serum devoid of any power to produce agglutination of bullock's erythrocytes was obtained. The experiments about to be referred to are recorded graphically on Plate II. As in previous experiments, the dose of normal rabbit serum (itself devoid of any action on bullock's erythrocytes) must first be determined.

	Amount in c.c.	Result
(1) Normal Serum	1.0	Trace
	0.75	? Nothing
	0.5	Nothing
	0.35	"
	0.25	"
	0.15	"

Control + 1 c.c. Saline = nothing.

Control for colour 0.25 Serum + 0.25 Saline.

This corresponds to Experiment I on Plate IV.

Serum of treated rabbit after heating to 56° C. for $\frac{1}{2}$ hour + 0.25 c.c. normal rabbit's serum and then + 1 c.c. of blood suspension	Undiluted	c.c.	Complete laking
		1.0	
		0.75	
		0.5	
		0.35	
		0.25	
		0.15	
	Diluted 1:10	0.1	
		0.075	
		0.05	
		0.035	
		0.025	
		0.015	
	Diluted 1:100	0.01	Diminishing
		0.0075	
		0.005	
		0.0035	
		0.0025	
		0.0015	
	Diluted 1:1000	0.001	
		0.00075	
		0.0005	
		0.00035	
		0.00025	
		0.00015	

Control + 1 c.c. Saline = nothing.

Control for colour 0.3 Serum + 0.3 Saline.

It has then to be ascertained that by heating for half-an-hour at 56° C. the serum of a treated rabbit (which had a strong power to produce laking) has been deprived of all action, also that it is possible to restore this action by the addition of normal rabbit serum, in a quantity which of itself is devoid of action. This is done as above, p. 43.

Experiments performed without the presence of normal serum show the heated serum to be quite an indifferent medium (see Experiment II and Experiment III, Plate IV). In Experiment III not 0·0025 of serum, the actual minimal laking dose, but a larger quantity, viz. 0·0035 c.c., was decided on for subsequent employment.

It had now to be determined what was the minimal quantity of normal rabbit's serum necessary to bring out the full action of 0·0035 c.c. of the heated immune serum. The employment in Experiment III (see Plate IV), of 0·25 c.c. of normal serum has been purely arbitrary, experience having, however, shown this to be a suitable quantity. The following experiment was therefore made. In each tube there was placed the above determined quantity of heated immune serum, 0·0035 c.c., and to it there was added in diminishing quantity normal rabbit's serum as follows:

		c.c.	
Normal rabbit's serum	Undiluted	0·3	Complete solution
		0·25	
		0·2	
		0·15	Diminishing to a trace
		0·1	
		0·05	
	Diluted 1:10	0·1	0
		0·075	
		0·05	
		0·035	0
		0·025	
		0·015	

Control + 1 c.c. Saline = 0.

Control for colour + 0·3 normal serum + 1 c.c. Saline.

It was decided to use 0·25 c.c. of normal serum and not 0·2 c.c. which the above experiment showed was the minimal necessary dose of normal rabbit's serum. The constant volumes of the different factors responsible for the haemolysis which are necessary for the experimental analysis of their relations to one another have now been determined.

(2) *Relations of the responsible factors to one another.* These preliminary determinations having been made, it was now possible to perform the following experiments on the behaviour of the three factors (viz. bullock's erythrocytes, the something normally present in rabbit's serum, and the new factor present in the latter after immunisation against bullock's corpuscles) to one another. We proceed in such a way that *one* of the latter two factors is placed in contact with the erythrocytes. Then, by centrifugalising the erythrocytes from the fluid, it is possible, by adding the factor previously omitted, to determine whether the factor first added has remained present in the centrifugalsed fluid or if it has passed to the sediment.

The Factor called forth by Immunisation.

(1) Of the heated serum of the immunised rabbit ten times the quantity, which it had been determined was adequate to effect complete laking of the 1 c.c. of blood suspension, was taken¹. To this was added 10 c.c. of blood suspension. The mixture was left in the incubator for half-an-hour and then centrifugalsed.

(2) There was thus obtained a sediment containing the erythrocytes and a clear fluid which was decanted, and with blotting-paper its last traces removed from the sediment. The erythrocytes and the rabbit's serum have thus been again separated.

At the commencement of the experiment the conditions had been 10 c.c. of blood suspension, plus 3.5 c.c. of 1:10 dilution of heated immune rabbit serum, *i.e.* a total of 13.5 c.c., therefore 1.35 c.c. contained a solvent dose of serum, and 1 c.c. of 5 % blood suspension.

(3) If now to the centrifugalsed fluid 0.05 c.c. of actual blood (not 5 % suspension) was added, these conditions were practically reestablished, provided that the sediment had not carried anything down with it before or during the process of separation from the fluid.

To the sediment there was added 13.5 c.c. of saline; then 1.35 c.c. was equivalent to 1.35 c.c. before centrifugalising. There remained the possibility that the constituents of the heated serum which had been poured off had been taken up by the erythrocytes before these were centrifugalsed.

(4) Of the centrifugalsed fluid the following quantities were taken, and to each was added 0.05 c.c. of blood (not 5 % suspension)

¹ In reality more than ten times the quantity determined in Experiment III, Plate IV, viz. 3.5 c.c. of 1:10 dilution.

and 0.25 c.c. of normal rabbit's serum, *i.e.* thus establishing the conditions necessary to laking of a second supply of bullock's erythrocytes, provided the fluid had remained unaltered.

c.c.	Result
2	0
1.75	0
1.5	0
1.35	0
<hr/> 1.25	<hr/> 0
1.0	0

The result as seen in Experiment V, Plate IV, was that the centrifugalised fluid had by contact with bullock's erythrocytes become an indifferent medium; for on the addition of normal rabbit's serum bullock's erythrocytes remain unchanged. The corresponding experiment with the centrifugalised sediment affords the explanation, and shows that whereas the fluid had lost the capacity to render the erythrocytes susceptible to the influence of the something present in normal rabbit's serum, the sediment had acquired this susceptibility, and in the acquisition had deprived the centrifugalised fluid of the constituent responsible for causing it. The sediment, plus the saline re-added as before described, was used in the quantity of 1.35 c.c. and normal rabbit's serum added in various quantities including also that quantity which preliminary determination of the constants had shown to be necessary to the complete laking of the 1 c.c. of blood suspension when 0.0035 c.c. of heated immune serum was present.

Sediment + saline		Normal rabbit's serum	Result
1.35 c.c.	+	0.3 c.c.	Complete laking
1.35 „	+	0.25 „	„ „
1.35 „	+	0.2 „	„ „
1.35 „	+	0.15 „	Not complete
1.35 „	+	0.1 „	Moderate
1.35 „	+	0.05 „	Slight

This corresponds to Experiment VI on Plate IV.

The quantitative conditions determined in the preliminary experiments had therefore been experimentally reestablished. The corpuscles had absorbed or otherwise used up the factor characteristic of the heated serum of an immunised rabbit in the proportion previously ascertained. By so doing they had become susceptible to the action not only of 0.25 c.c. of normal rabbit's serum, but to the actual minimal dose (*viz.* 0.2 c.c.) of this serum necessary to bring into effect

the action of the previously determined minimal dose of heated immune serum. The assumption of the existence of special affinities between the bullock's erythrocytes and the factor induced to appear in the serum of the rabbit by their previous injection therefore appears to be fully justified. It will now be legitimate to designate this factor as an "anti-erythrocytic body" directed against the cells (erythrocytes) responsible for calling it into existence.

The Factor normally present in Rabbit's Serum.

The something normally present in rabbit's serum is not quite indifferent¹ to bullock's corpuscles, but this relative indifference disappears when the latter have previously been subjected to the influence of the specific "anti-erythrocytic body." When the experiments just described are repeated with the normal rabbit serum the results for centrifugalised fluid and corpuscles are exactly the opposite of those obtained above. The fluid apparently loses none of its properties, and the bullock's erythrocytes which have been subjected to the action of the normal fluid, appear to have acquired no property that distinguishes their behaviour from normal bullock's erythrocytes.

(1) 10 c.c. of blood suspension and 2.5 c.c. of normal rabbit's serum gives a total of 12.5 c.c. in 1.25 c.c. of which the necessary constant dose of normal serum and 1 c.c. of 5 % blood suspension is contained.

The mixture remained half-an-hour in the incubator and was then centrifugalised.

(2) After centrifugalising the fluid from the sediment, they were tested separately.

(3) The factor omitted at the outset, viz., 0.0035 c.c. heated serum of immunised rabbit, was used to test the behaviour of the factor normally present as follows.

(4) The centrifugalised fluid was taken in the following quantities, and to each tube was added 0.35 c.c. of 1:100 dilution of the heated immune serum, and 0.05 c.c. of actual blood, so that the experimental conditions for the interaction of three factors were established.

Centrifugalised fluid		Heated immune serum		Blood (not suspension)	Result
2.0 c.c.	+	0.0035 c.c.	+	0.05 c.c.	Complete laking
0.75 "	+	"	+	"	" "
1.5 "	+	"	+	"	" "
1.35 "	+	"	+	"	" "
1.3 "	+	"	+	"	" "
1.25 "	+	"	+	"	" "

¹ Cf. also Robert Muir, *loc. cit.*

The normal serum contained in 1.25 c.c. of the centrifugalised fluid had therefore lost nothing by contact with the bullock's erythrocytes. This corresponds to Experiment VII on Plate IV.

To the sediment was added the necessary amount of saline for 1.25 c.c. to again contain 1 c.c. 5% blood suspension, and the factor previously absent, viz., the heated immune serum, was added in the quantities stated.

Sediment + saline		Heated immune serum		Result
1.25 c.c.	+	0.01 c.c.		Nothing
1.25 „	+	0.0075 „		„
1.25 „	+	0.005 „		„
1.25 „	+	0.0035 „		„
1.25 „	+	0.0025 „		„
1.25 „	+	0.0015 „		„

The factor present normally in rabbit's serum had therefore the appearance of being quite indifferent to the bullock's corpuscles under these experimental conditions. The corpuscles had not absorbed any of it, for had they done so they must have been dissolved in the presence of the excess of heated serum of the immunised rabbit. This corresponds to Experiment VIII on Plate IV. It would lead into a very theoretical digression to discuss whether the results of the foregoing analysis are dependent upon the special experimental and quantitative relations of the factors present in normal and in immune rabbit's serum.

On the basis of the experiments which have been detailed in the preceding pages, one is justified in regarding the body present in the serum of an immunised rabbit as a body of an antitoxic nature. This is the main point.

I do not wish to discuss whether the something present in normal serum of rabbit acts *directly* on the erythrocytes, which have been subjected to what has been regarded as the mordant-like influence of the anti-erythrocytic body, nor whether the factor normally present in serum unites with the anti-erythrocytic body, the latter being regarded as an intermediate body linking on the factor of normal serum to the erythrocytes. The production of the antitoxic body and the readiness of estimation of the degree of its production are the points of interest, at present. The accident that with the assistance of a second factor normally present in serum, laking of the erythrocytes can be produced *does not affect this paper*, except in so far as normal serum has been employed as an indicator of the modification produced in erythrocytes which have been acted upon by an immune serum. It is not proposed to discuss the theoretical explanations of the mechanism of haemolysis.

VI. *The Course and Progressive Augmentation of Artificial Immunity.*

The consequences of injecting erythrocytes illustrate exceptionally well some of the features in the course and progressive augmentation of acquired immunity. As in the case of a toxine, the primary injection is followed by an interval within which immunity is being acquired, but is usually not demonstrable. Some of the phenomena of immunity soon become demonstrable, and from day to day they become more strongly developed till a maximum is reached. Thereafter, constancy is maintained for a varying period, and ultimately a gradual diminution sets in. The acquisition, rise, and constancy of the haemolytic phenomena following upon the injection of erythrocytes are well shown on Plates V and VI, on which the experimental determinations made at different dates are succinctly recorded. The comparison of the actions at different dates shows the sudden acquisition, the gradual rise, and the constancy of the haemolytic power. These plates also show that further injections of erythrocytes are followed by succeeding augmentations¹ of the haemolytic power, which resulted from the primary injection. In this way the immunisation process may be pushed in order to obtain a serum characterised by great haemolytic or antitoxic powers. The haemolytic powers of immune serum in its natural state are recorded on Plate V, and it will be noted that the curve obtained is different from that shown on Plate VI, on which the haemolytic powers of the heated immune serum are recorded when a constant quantity of normal rabbit's serum has been added to make the haemolysis effective. The difference in the two curves is important. It is due to the fact that the immune serum in its natural state has only acquired anti-erythrocytic body, and has not had any excess of the factor naturally present superadded by immunisation. This latter factor has nothing whatsoever to do with the acquisition of immunity, of which it is quite independent, and for the purposes of these experiments its addition, as in Plate VI, fulfils solely the function of making the erythrocytes effective indicators of the presence of the anti-erythrocytic body. The erythrocytes together with the factor naturally present in the serum form a kind of compound indicator of the presence of anti-erythrocytic body.

¹ Immediately following the injection there is a transitory fall in haemolytic or antitoxic power (see Bullock, *Trans. Path. Soc. London*, 1902).

How is the Progressive Augmentation Artificially Produced?

It must be borne in mind that the haemolytic or antitoxic powers which the serum of an artificially immunised animal may acquire are only some of the manifest accompaniments of immunity, and not its cause. When a certain amount of blood or a small dose of toxine is injected into a highly immunised animal, the immunity is increased although the serum of the animal would be expected to at once abolish the effect¹ of the injections.

Outside of the body 1 c.c. of the immune animal's serum may be able to account for many multiples of the amount of blood, or the toxic effects of many times the dose of toxine, and yet notwithstanding what must be the much greater potency of the total amount of serum in the body, small doses of corpuscles or of toxine are still effective, immunising agents.

It is easy to allow bullock's erythrocytes to load themselves with the anti-erythrocytic body in an immune rabbit's serum until they are unable to take up any more. If this be done before the erythrocytes are injected into an immune rabbit, one may suppose that the process of neutralisation assumed to occur when injections are practised on immune animals, has been imitated outside the body.

We will also be justified in assuming that the supposed neutralisation within the body really does occur, if erythrocytes after saturation with the anti-erythrocytic body *in vitro* give rise to an augmentation in the immunity of an already immune animal. As a matter of fact, erythrocytes which have been loaded with anti-erythrocytic body² produced the augmentation in immunity which is demonstrated by the records on Plates Nos. V and VI. The conclusion from these observations is obviously that augmentation of the immunity to erythrocytes occurs notwithstanding the probability that when injected into an immune rabbit the serum of the latter will neutralise them. But this neutralisation involves retention, by the resulting product, of those features which in the unneutralised erythrocyte are responsible for the production of the anti-erythrocytic body.

¹ The toxic effects of multiples of the lethal dose are abolished, and it is perhaps loose reasoning to assume by analogy that all effects will be abolished, for obviously they are not.

² Experiments in which such saturated erythrocytes were used for the primary injection yielded positive results in 6 out of 8 cases. The haemolytic power was, however, in three instances less than in the controls in which the same quantity of erythrocytes in their natural condition was injected. The reasons for this difference seem obvious. The anti-erythrocytic body produced is identical in both cases.

In view of the theoretical importance of these conclusions, it will be well to state that pains were taken to exclude fallacies, and that the same result was obtained (1) when the immune serum used to saturate the erythrocytes was obtained from one rabbit and the "neutralised" erythrocytes were injected into the peritoneum of another immune rabbit.

(2) When the immune serum was derived from the rabbit into which the injection was made.

(3) When the immune serum was derived from the animal into which the injection was made, the primary immunity obtained by subcutaneous injection, and the injection of "neutralised" erythrocytes was made into the peritoneum.

Under these circumstances there could be no question of the conference of passive immunity by means of a more highly haemolytic serum.

Summary of Haemolysis Experiments.

From the foregoing repetition of the experiments of Bordet and Ehrlich and Morgenroth, the conclusion is now drawn that by a process of immunisation, in the case of rabbits, a factor directed against the erythrocytes of the bullock is produced, and that this factor is in a wide sense of the term of an antitoxic nature, and in a narrower sense an "anti-erythrocytic body." The only real phenomena which are the concomitant consequences of acquired immunity are the production of the agent or agents directed against the erythrocytes and their reaction with the latter. The erythrocytes which have taken part in this reaction are laked in the presence of an agent present normally in serum, and we are thereby enabled to detect the existence of one of these concomitants. This seems to me to be the important fact, and without discussing the conflicting hypotheses bearing on the mechanism of the process, I take it as established that a means for exactly quantitatively estimating this antitoxic, or more correctly, anti-erythrocytic body, is afforded by the addition of a constant quantity of the serum of a normal rabbit¹.

¹ It is to be noted that throughout these experiments the normal serum used has been obtained from the same species as the immune serum, viz., the rabbit. It is customary to employ the normal serum of the guinea-pig. I used normal rabbit's serum to obviate introducing new factors in the conditions of experiment and consequently possible fallacies. The relations of the quantities of the immune and normal sera necessary to effect laking may be made to vary—a larger proportion of heated immune serum requires a smaller quantity of normal serum and *vice versa*.

This holds good even if the presence of the serum of a normal rabbit do no more than provide conditions favourable to the solution of the membrane of the modified erythrocytes. Nor does it matter whether the anti-erythrocytic body has chemically united with constituents of the erythrocytes to provide new bodies soluble in the presence of normal serum, or combining to form products soluble in the constituents of normal serum. Even if, as some hold, the membrane of the erythrocytes is simply ruptured in consequence of its being no longer able to resist osmotic pressure previously withstood, the procedure is reliable for the estimation of the extent to which one concomitant feature has been developed in the immunity attained by an animal immunised against erythrocytes.

VII. *Conference of Passive Immunity to Erythrocytes.*

The basis of serum therapeutics is that in suitable cases the injection into another individual of the serum of one actively immune, confers on the former some of the characteristic accompaniments of the latter's immunity. The anti-erythrocytic serum of the rabbit readily confers its power on the serum of rabbits or guinea-pigs into which injections of this serum have been made. The passive acquisition of anti-erythrocytic power may be demonstrated without difficulty by the methods previously recorded, if the sera be examined soon after the injection. After a short lapse of time the power thus conferred is lost. In the rabbit it appears to gradually diminish without any attendant phenomena: but in the guinea-pig (*i.e.*, in a species different from that which produced the anti-erythrocytic body) the decline is accompanied by another phenomenon, *viz.* the appearance of agencies directed against the foreign anti-erythrocytic body injected. The demonstration of this phenomenon for erythrocytes is a somewhat complicated process and need not be detailed here, because in the case of ricin the experimental graphic demonstration is a much simpler process, and will subsequently be alluded to.

Immunity to Toxines.

As erythrocytes cannot be regarded as toxines in the strict sense of the term it is important to ascertain whether or not the phenomena described for acquired haemolysins are also characteristic of acquired immunity to the toxines proper. For this purpose, experiments have been performed with ricin, croton, cobra venom, diphtheria, and tetanus toxines. The investigation has been most extensive for ricin. Ricin lends itself well to graphic demonstrations, and therefore the following topics are discussed with more especial reference to immunisation against ricin.

VIII. *A Mixture of Toxine and Antitoxine physiologically neutral in vitro, is also physiologically neutral in vivo*¹.

It is well known that ricin clumps the red corpuscles of the rabbit, and that the serum of a rabbit immunised against ricin possesses the power of abolishing this action by virtue of an antitoxine, which it is customary to call antiricin. The toxic effects of ricin on the living animal have nothing to do with its action on the red blood corpuscles, which in the following experiments are merely used as indicators.

The current belief that if a mixture of toxine and antitoxine be neutral *in vitro*, it will also be innocuous *in vivo*, is based on an experiment published by Ehrlich in 1898, at a time when the lethal action of ricin was believed to be due to clumping of the red blood corpuscles, and consequent thrombosis. I have been able to fully confirm Ehrlich's conclusions; but venture to qualify them by adding that the statement which heads this paragraph is only rigidly applicable to the one species of animal which has yielded the antitoxine and serves for the determination of the lethal dose. In Ehrlich's experiment erythrocytes were used to indicate where free ricin was present in a series of mixtures of ricin and antiricin; then a similar series of mixtures was tested on animals, and the conclusion was arrived at that the erythrocytes gave reliable indications of the physiological neutrality of such mixtures for living animals.

I have repeated Ehrlich's experiment in a different and more detailed manner. It was possible to devise a procedure whereby in a series of test-tube experiments the minimal agglutinating dose of ricin for erythrocytes was so adjusted that this quantity was also the minimal lethal dose for a rabbit of two kilogrammes, if subcutaneously injected. Therefore, in the experiments which follow on the relations between ricin and antiricin *in vitro*, these relations are also equally those which obtained when corresponding mixtures were injected into living rabbits. What is spoken of as complete clumping in the test-tubes is equivalent to death in a rabbit of two kilogrammes, and lesser degrees of clumping correspond to diminished degrees in the toxic effects of the ricin. In

¹ This is merely stating in other terms the experimental observations necessary for the study of the progressive augmentation of immunity to erythrocytes recorded on p. 49. A physiologically neutral mixture of toxine and antitoxine can only be determined with a large margin of error. I explained this matter fully in a paper on "The qualitative and quantitative relations of toxine and antitoxine" (*Lancet*, Oct. 17, 1903). For the purposes of immunisation by "neutralised" toxine or erythrocytes, I avoided an obvious fallacy by using more antitoxine than the erythrocytes indicated was necessary.

the same way complete abolition of clumping corresponds to complete abolition of the toxic effects, and imperfect abolition of clumping to diminution in toxicity.

*Determination of a Physiologically Neutral Mixture
of Toxine and Antitoxine.*

(a) *The Unit Dose of Toxine.* (See Plate VII.) In a series of test-tubes, all containing the same total volume of fluid, with equal quantities of blood, but progressively diminishing quantities of ricin¹, it is possible to determine the smallest quantity of ricin which causes complete clumping of all the blood corpuscles. The experimental details are similar to those for haemolysis: 1 c.c. of a 5% suspension of rabbit's erythrocytes which had been freed from their serum by washing, was used throughout. With adequate quantities of ricin the corpuscles may be caused to adhere to the wall of the test-tube as well as to one another, thus leaving the suspending fluid quite limpid. With lesser quantities of ricin the clumping diminishes and an increasing proportion of the corpuscles remains suspended in the fluid after the tubes have been shaken. It therefore follows that if equal portions of fluid be withdrawn from such a series of tubes and allowed to diffuse themselves on blotting-paper, the phenomena visible in the test-tubes will be transferred to a series of circles on the blotting-paper. It will be convenient for the purposes of demonstration to concede that within these circles corpuscles will be absent, if there be complete clumping. They will be present, and the extent and uniformity of their diffusion will increase as the degree of the clumping diminishes. As the quantitative relations are stated on Plate VII they need not be detailed here.

(b) *Determination of the Antitoxine equivalent of the Unit Dose of Toxine.* (See Plate VII.) Similarly, if a constant quantity of anti-ricin serum be added to a series of test-tubes otherwise identical with those above referred to, the extent to which the corpuscles diffuse after transference of samples of the fluid to blotting-paper will vary directly with the extent to which the action of the ricin has been abolished.

Thus on Plate VII, series 1, it is evident that down to tube 9, in the series, there was complete clumping, and that lower down the series the clumping diminished inversely as the diffusion of the corpuscles

¹ Exact figures are stated on Plate VII, and full details of similar experiments will be found in a paper in the *Journal of Pathology and Bacteriology*, Dec. 1903.

increased, till a degree of diffusion was reached as great as that manifested by corpuscles merely suspended in saline.

In series 2, uniformity in the diffusion of the corpuscles is present much higher up the series: for the action of the ricin was abolished by antiricin, complete abolition being effected up to tube 6, where the diffusion is as uniform as in the control at the end of series 1. Higher up the series the diffusion becomes less and less uniform as the degree of clumping increases and the action of the antiricin diminishes.

For toxines such as those of diphtheria and tetanus the above determinations can only be made on living animals. The principle is, however, the same.

IX. *Demonstration that the Antitoxine is specific for the species in which it is produced.*

The results of the two preceding experiments may now be reproduced at will, for one can be sure of (*a*) the quantity of ricin which will always completely clump a standard quantity of blood, and (*b*) of the quantity of antiricin serum which will as surely prevent this action. In a series of tubes in each of which this equilibrium between ricin and antiricin has been established, it is possible to study the result of the introduction of any new factor into the experimental conditions. If a new factor added act upon the antiricin so as to deprive it of its power to abolish the action of the ricin, the latter will show that this has occurred by reproducing under the new conditions clumping of the corpuscles similar to that in series 1, Plate VII.

In all the tests represented in series 4 (Plate VII) constant quantities of ricin, antiricin and blood were present in the proportions needful for complete absence of clumping as determined above. The clumping in this series has been caused to appear under new conditions by the addition of the serum of a guinea-pig which was in a late stage of passive immunity. The serum of the passively immune guinea-pig was added in quantities increasing from left to right, and it will be noted the clumping is less perfect on the right-hand side. The passive immunity has been conferred on the guinea-pig by injecting the serum of an actively immune rabbit, and as the passive immunity declined, the serum of the animal acquired the peculiarity here demonstrated. This is really a reaction directed against the antitoxine, which although innocuous to the guinea-pig, is still dealt with as an agent foreign to its constitution.

When a new factor is introduced, it is also necessary to perform control experiments¹ designed to exclude fallacies, and especially such as may be introduced by the production of similar results by different mechanisms, *e.g.*, in this case the production of clumping by the two sera in the absence of ricin. Further, when one studies the influence of increasing quantities of the serum of a passively immune guinea-pig, on the above determined constant quantities of ricin and antiricin, it is necessary to control the observations by a similar series of observations in which normal guinea-pig serum replaces the serum of the passively immune guinea-pig in order to be able to be sure that the modifications produced are peculiar to passive immunity. Normal guinea-pig serum is shown to be indifferent to antiricin, which in its presence is quite able to abolish the action of ricin.

When rabbits have been rendered passively immune the phenomenon obtained for guinea-pigs does not present itself, or more correctly, I have not been able to detect its occurrence. It therefore seems possible that the antitoxine was specific for the species of animal in which it was produced.

The same phenomena can be demonstrated when a haemolytic immune serum of a rabbit is used to confer passive immunity on rabbits and guinea-pigs.

X. *Interference of Normal Rabbit's Serum with the Agglutinating Action of Ricin.*

The addition of normal rabbit's serum prevents the proper manifestation of the agglutinating action of ricin. In the test-tube reaction there is nothing to show that this interference is not identical with that exercised by the antiricin serum of an immunised animal. It appears to differ only in degree (*cf.* Plate VII, series 2 and 3). My experiments were so devised that the minimal agglutinating dose in the test-tube was also the minimal lethal dose for a rabbit. It was therefore an easy matter to determine that the interference of normal serum with the agglutinating action *in vitro* did not correspond to abolition of toxic action *in vivo*. For this purpose the quantity of normal serum which completely abolished the agglutinating action *in vitro* was taken and its action tested *in vivo*. The normal serum did have an influence upon the action of ricin when the latter was subcutaneously injected,

¹ The control experiments are discussed fully in a paper appearing in the *Journal of Pathology and Bacteriology*.

but at most this only amounted to diminution, and in some instances to abolition of the local necrotic action of ricin. The serum was not able to prevent the development of constitutional symptoms. This result showed that the interfering action of normal serum in the test-tube was not due to a quantity of "natural" antiricin too small to be effective, for in the latter case in experiments on the living animal it is the constitutional and not the local effects of ricin which are diminished. The interfering action of normal serum is therefore not due to naturally present antiricin, and in the acquirement of immunity to ricin, the antitoxine (antiricin) is something superadded to the serum of the immunised animal.

XI. *Augmentation of the Acquired Immunity to Ricin.*

Experiments similar to those recorded in connection with the course and augmentation of immunity to erythrocytes were performed with ricin. Erythrocytes can be used to determine that one has a physiologically neutral mixture of ricin and antiricin. With such mixtures experiments parallel to those performed with erythrocytes saturated with anti-erythrocytic body were made, and yielded confirmatory results. The power of the immunised animal's serum to neutralise ricin was increased when such mixtures were injected into an already immune animal, and therefore the reaction between ricin and antiricin had not modified the properties of the former in so far as its capacity to lead to the production of antiricin was concerned. It was also found that rabbits which had repeatedly received physiologically neutral mixtures of ricin and antiricin (derived from rabbits) slowly acquired immunity to ricin.

SUMMARY.

By means of the graphic records given on Plates II—VI and VIII the following facts have been illustrated.

Immunity to Erythrocytes.

Normal rabbit's serum is relatively innocuous for bullock's erythrocytes.

The serum of an immunised rabbit acquires the power to dissolve bullock's erythrocytes.

Besides acquiring the power to dissolve bullock's erythrocytes, an

immune serum may also acquire power to clump them, and it has been shown that the phenomena of haemolysis and of agglutination are independent.

The powers acquired by the immune serum can be artificially modified. The serum may be deprived of its powers by heat. Serum cautiously so deprived of its haemolytic power can have it restored by the addition of normal serum. The haemolytic power of the unheated serum is augmented if normal serum be superadded.

It has been shown that an immune serum only differs from a normal serum by its containing antitoxic bodies which are endowed with powers of specific reaction with the bullock's erythrocytes.

The mechanism by which erythrocytes are laked by an immune serum has been analysed, and it has been shown that the solution of the erythrocytes is effected through the intervention of an anti-erythrocytic body called forth by immunisation. The erythrocytes which have been subjected to the action of this product of immunity give indication of their reaction with it if they are subsequently or concomitantly placed under the influence of normal serum. The erythrocytes and normal serum together, therefore, form a combined indicator of the presence of the anti-erythrocytic body. The part played by normal serum has nothing to do with the acquisition of immunity.

The only conclusion drawn from the above observations is that in the production of immunity to erythrocytes the serum of the immunised animal acquires certain powers which are concomitant with, but are not necessarily the cause of the immunity. This special case of immunity to erythrocytes is therefore probably parallel to induced immunity to those bacterial toxins for which antitoxines are known to exist.

The course and progressive augmentation of artificial immunity to erythrocytes has also been illustrated, and it has been shown that erythrocytes saturated with anti-erythrocytic body retain the power to augment the immunity of an already immune animal.

The serum of an animal actively immunised has power to confer passive immunity upon other animals, and the course of this passive immunity differs in the two cases when it is induced in the same species and in a species alien to that providing the immune serum.

Immunity to Ricin.

The experiments with bullock's erythrocytes have been repeated in parallel observations with ricin in order to permit of the observations on haemolysis being utilised in drawing conclusions on the behaviour of bacterial toxins.

By adjusting the conditions of experiment in such a way that the minimal lethal dose for an animal was also the minimal agglutinating dose in test-tube experiments, it has been possible to give graphic records showing the parallelism between the processes when erythrocytes or living animals are used as indicators of the presence of free ricin. In this way it has been possible to illustrate the determination of the minimal lethal and minimal agglutinating doses of ricin and that quantity of antitoxine (antiricin) which is necessary to abolish the corresponding actions in the animal and in the test-tube, and to show that the mixture of toxine and antitoxine which is physiologically neutral *in vitro* is also physiologically neutral *in vivo* within the limitations imposed by the preliminary determinations.

The consequences of conferring passive immunity upon the guinea-pig by means of active immune serum of the rabbit have also been illustrated, and it has been shown that the alien antiricin serum leads to the production of agencies directed against itself.

Ricin neutralised by antiricin retains its power to produce immunity when injected into the species of animal which has yielded the antiricin.

In connection with the conference of immunity to erythrocytes and to ricin, the nature of the difference between normal and immune sera has been studied. Attention has been directed to the possession by normal sera of properties which simulate those possessed in more marked degree by the immune sera. In the case of haemolysis, it has not been possible to clearly demonstrate that the actions manifested by the normal and immune sera are distinct, although the weight of evidence is in favour of this view. In the case of ricin, however, it has been possible to demonstrate that the immune serum possesses properties which are quite distinct from those possessed by normal serum, and that the latter does not interfere with the action of ricin because of the natural presence of a trace of antiricin. In the case of immunity to ricin, the antitoxine is certainly something which has been super-added to the serum in consequence of the process of immunisation.

The facts ascertained in regard to artificial immunity to erythrocytes and to ricin completely agree. Only in one point is it impossible to be quite sure that the phenomena are identical, viz., in the simulation by normal serum of the powers characteristic of the immune serum; for the demonstration that the two are distinct has been possible for ricin, but open to doubt in the case of erythrocytes. My investigations have been extended to diphtheria and tetanus toxins and to cobra venom, kindly placed at my disposal by Sir Thomas R. Fraser. They have however been interrupted, but so far as they go they support fully the observations made on ricin and erythrocytes.

SOME GENERAL CONCLUSIONS.

The following summary emphasises some points of importance, in a manner which is apt to give to the statements contained therein a definiteness which the results of further investigations may not justify. A subjective element necessarily enters into our judgment of the significance of these complex phenomena.

I. The acquirement of antitoxic power by the serum of an immune animal has been variously explained. The antitoxine may be regarded as a something normally present in the organism, but by a process of immunisation quantitatively increased (Ehrlich's side-chain theory); the antitoxine may be regarded as a new product, *i.e.* as a something superadded to the organism subsequent to the introduction of toxine into it, the mechanism responsible for its production has been differently conceived by Buchner, Fraser, and Metchnikoff. Professor von Behring holds a somewhat intermediate position by assuming that an antitoxic serum differs only from a normal serum in that the albuminous constituents of the latter have acquired a "force inseparable from them, and comparable to the conference of magnetism on previously non-magnetised iron." In my opinion the balance of evidence is in favour of the antitoxine being something superadded to the immune animal.

It is known that normal serum interferes with certain reactions manifested by various bodies *in vitro*. The bodies referred to are antitoxine-producing and not antitoxine-producing. For some bodies, which do not produce antitoxines, I have demonstrated that no increase in this power of serum to diminish a reaction can be obtained¹. We have

¹ Further details are given in the *Journal of Pathology and Bacteriology* (March, 1902) and in the *Archives de Pharmacodynamie* (T. VIII, 1900).

no right to assume that antitoxine is naturally present in certain animals. The interfering power of serum *in vitro* is not explained by postulating the presence of antitoxine naturally. To prove that the power possessed by normal serum *in vitro* is truly antitoxic, it must be shown that the supposed antitoxic power *in vitro* corresponds to the abolition of the lethal action or an equivalent diminution of the lethal power *in vivo*. This I have found not to be the case. This power of serum *in vitro* has only the appearance of being an antitoxic reaction, it is really a pseudo-antitoxic reaction, and cannot be due to normally present antitoxine.

II. I cannot pretend to assert that the antitoxine is derived from the toxine. I can only advance the following results of experiment as having a bearing on the discussion of what is the source of antitoxine.

As shown in this paper, in the same species as that producing the antitoxine, union with the antitoxine does not abolish the efficacy of the toxine as an antitoxine-producing agent; therefore, under these circumstances, the antitoxine probably does not destroy the toxine, and in the combination (toxine and antitoxine) the toxine exists as such, and a simple explanation is afforded of how it comes about that in a highly immune animal the injection of small doses of toxine leads to further rises in the degree of immunity, although one cubic centimetre of the serum of such an immune animal would neutralise the toxicity of many multiples of the quantity of toxine injected. The view that the antitoxine does not destroy the toxine is no new one; Ehrlich has long held that the two unite with one another in a manner analogous to the formation of a double salt, but I believe these experiments give this view an amount of support which other investigations have failed to do. All sorts of hypothetical heightened affinities of cells, or cell "side-chains" for toxine; of liberation of toxine, allowing the latter to reach the cells; of slow combination of toxine with antitoxine, etc., have been advanced to explain the phenomenon that a small quantity of toxine injected into an immune animal leads to a further increase in the antitoxic power of this animal's serum. All this appears to me to be explained easily as above, assuming that both toxine and antitoxine retain their individuality in the species of animal producing the antitoxine, and that the latter is probably not lost to the organism in exercising its antitoxic function. It is a well-recognised fact that the early stages of those forms of artificial immunity which are accompanied by the appearance of antitoxine, are the most difficult to obtain, and

various devices, preliminary stomach administration, modification of the toxine by chemical means, are resorted to. If the early stages of immunity be once attained the production of further immunity proceeds without difficulty, and it may well be that the earliest and most difficult phase of immunity to attain is the production of antitoxine due to the presence of toxine alone, and that, in the later phases, toxine combined with antitoxine is the important factor in the production of the progressively increasing immunity.

In a species other than that which has yielded the antitoxine the toxine and antitoxine appear to have lost their individuality. The combination (toxine and antitoxine) may here react as one body, and its injection into a different species from that which yielded the antitoxine may lead to no production of antitoxine.

The fact that toxine neutralised by antitoxine can call forth both the earlier and the subsequent stages of immunity with antitoxine production has, however, another consequence of far-reaching importance. The strongest argument in support of the view that an antitoxine is a special secretion of cells, loses its significance if toxine neutralised by antitoxine can produce immunity. It has hitherto not been possible to meet the argument that the amount of antitoxine produced ought to be in proportion to the amount of toxine injected if the former is derived from the latter. It has been argued, that, because the amount of antitoxine produced has exceeded the amount of toxic bouillon injected (as estimated in terms of the number of lethal doses contained, in the ratio of, for example, 1 : 20,000 as found by Professor Woodhead), it was inconceivable that the antitoxine could be formed from the toxine. The number of lethal doses contained in a given quantity of toxic bouillon yields, however, no criterion of the antitoxine-producing capacity of the bouillon. The degree of antitoxine production is proportional to the sum total of the quantities of toxic bouillon injected, or more accurately, the greater the sum total of toxine injected, the greater is the amount of antitoxine produced. The results I have obtained with fully neutralised toxines demonstrate the utter unjustifiability of the above argument. In my cases, in terms of the toxicity introduced and antitoxine produced, the ratio has been as 1 to infinity. I hold it, therefore, to be established that it is erroneous and inadmissible to require that if the antitoxine be formed from the constituents of the toxic bouillon the ratio of the production of antitoxine should correspond to the estimated number of lethal doses of toxine injected. My remarks in this connection are,

of course, only based on the consequences of injecting toxine neutralised by antitoxine into the species of animal from which the antitoxine was derived and when the minimal lethal dose of toxine has also been determined on the same species.

There is, however, another matter which requires consideration in this connection. On the assumption that a toxine may suffer diminution in its poisonous properties without its capacity to unite with antitoxine being in any way diminished, there has been based the conception that in the toxine molecule there are present two independent atomic groups. One of these groups is regarded as being responsible for the production of the toxic symptoms, the other group is regarded as the one which combines directly with side-chains of the cells suffering under the poisonous action. It is alleged that it is only through the intermediation of this second atomic group that the toxic group comes to be able to exercise any influence whatsoever on the cells. In accordance with these conceptions it is held that a toxine, while losing its toxicity, can retain its power to neutralise antitoxine, and the inference is drawn that the two hypothetical atomic groups are absolutely independent of one another. Further, because a toxine which has become diminished in toxicity may still retain its power to lead to the production of antitoxine, it is concluded that the atomic group held to be responsible for the production of the poisonous action has nothing whatsoever to do with the production of antitoxine, and that therefore the latter is solely the consequence of the action of the other atomic group. The atomic group which is regarded as being responsible for the manifestation of toxic action, has been designated *Toxophore*. The atomic group which has been held solely responsible for the union with the "side-chains" of cells, has been designated *Haptophore*.

When a quantity of toxine is injected into an animal, we are only able to estimate the quantity by stating that a certain number of lethal doses has been injected. That is, adopting the phraseology of the above hypothesis, we estimate the quantity of toxine injected in terms of the *toxophore* group. When we estimate the power of a serum to neutralise toxine, we estimate its antitoxic power in terms of the *haptophore* group. According to the hypothesis explained in the preceding paragraph, the antitoxine is really an *antihaptophore*, and not an *antitoxophore* atomic group, and its underlying principle at the outset is that the *toxophore* group has nothing whatsoever to do with the *haptophore* group of the toxine, yet in estimating the amount of antitoxine produced we find ourselves actually estimating the one group in terms of the other. For, supporters of this view have demanded that if the antitoxine, or rather, *antihaptophore* group be a derivative of the toxine the quantity produced should be in direct proportion to the amount of toxine or, rather, *toxophore* groups injected, and in estimating the amount of antitoxine or, rather, *antihaptophore*, which is produced in consequence of an injection of toxine, it is their custom to state that the amount is equivalent to so many lethal doses or, in the special phraseology adopted, equivalent to so many *toxophore* groups. Obviously, there is a grievous confusion of thought in such a method of conceiving the relations obtaining here. At the outset, on injecting a certain number of lethal doses, the number of lethal doses which we have injected will give us, according to

this conception (that the toxophore and haptophore groups stand in no relation to one another), absolutely no idea of the number of haptophore groups which have been injected.

These considerations make it essential to more fully enquire into the extent to which the poisonous properties of a toxine may diminish, and its antitoxine neutralising power nevertheless remain constant ; it will also be well to reinvestigate the power of modified toxines to lead to the production of antitoxine, and to exclude the possibility that this modification of a toxine is not a stage in the formation of the antitoxine. Knorr's experiments with tetanus toxine seem to indicate the proper line of enquiry in this connection.

III. I have adduced evidence pointing to antitoxines being specific in two distinct senses:

(1) As regards the toxine leading to its production.

(2) As regards the species in which it is produced.

These conceptions of a double specificity are intelligible if the antitoxine be a new product not present in an immunised animal previous to the injection of toxine. For if the antitoxine be a product derived from the toxine through the intermediation of cell activity, to some extent after the well-known views of Metchnikoff, or in the sense of Buchner or of Fraser, then the specificity in regard to the species of animal is explicable, as is also the specificity in regard to the toxine, because the power to react with the mother toxine will be the basis of the antitoxic function ; and the divergence in the character of the antitoxines of differing species is certainly no more remarkable than the very marked divergence in the character of the different albuminous constituents of the sera derived from identical nutriment by differing species.

In conclusion, if the antitoxines have a common cause of origin, viz. the toxine, and have also the common property to neutralise the toxine, yet differ among themselves, *a priori* there would seem little objection to the hypothesis that the common cause of their origin should itself be the source, and that the antitoxine is in some way derived from the toxine. While making this statement I do not advance it as my belief, the evidence as yet by no means justifies its acceptance ; the following considerations, however, give to it still further support.

IV. Our ideas on immunity have become to a large extent mere generalisations based on the evidence derived from special cases, *e.g.* diphtheria, tetanus, snake-poisoning. The study of other toxic diseases shows that different conditions may exist in these. The assumption which has for so long dominated our conceptions in this

field, viz., that *all bacterial* toxins must of necessity produce antitoxines, and that we only require to devise suitable means to obtain the latter, has tended to confine investigation to one narrow groove, and seems to me to have been a barrier to progress. The organism long ill of tuberculosis, acne, carbuncle, has, for example, lasting opportunity to produce antitoxine to the poisons of the tubercle bacillus and staphylococcus. Have we any evidence that the organism does so? In all sorts of new positions new foci of tubercular material may arise, during a long period of years many new acne pustules may develop. When healing occurs the evidence all points to this being a local process, as in the case of erysipelas. It is needless to multiply instances, in which a toxine appears to play a part in producing symptoms of illness, which do not, however, abate on account of anything of the nature of a true antitoxic reaction. Of all diseases tuberculosis seems to demonstrate that a patient in an advanced stage is able to support and deal with the products of unnumbered millions of tubercle bacilli, such a number as in an earlier stage of his illness would, if suddenly presenting themselves, have surely produced an acutely fatal disease. Is this relative degree of immunity due to antituberculin or other antitoxine? I am not aware that this idea has ever been entertained, and there is certainly nothing to justify it. The evidence does not justify the assumption that *all bacterial* toxins must of necessity produce antitoxines; and if the latter be products derived from the toxins, it may well be that, if the metabolic products consequent on the presence of the toxins in the organism be unable to react with the mother toxine, there is no basis for the antitoxic reaction. It seems not impossible that the generalisation from a few special cases of immunity will prove to have been unjustified, and that each case will have to be regarded from its own standpoint till our knowledge is much more extensive.

Finally, I desire to express my thanks to Dr G. H. F. Nuttall and Mr J. A. Murray for the advice and active assistance which has made the publication of this article and the preparation of the plates possible at this time. The work was performed chiefly in the laboratory of Sir Thomas R. Fraser, but also to some extent in that of Professor Oscar Liebreich, to them and also to Professor W. S. Greenfield I desire to acknowledge my indebtedness. The expenses incurred in connection with the illustrations accompanying this paper have been mainly defrayed by a grant from the Moray Fund for Research of the University of Edinburgh.

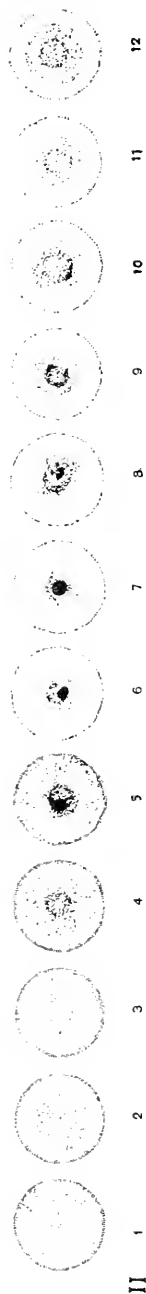
PLATE II. illustrates in detail the experiments figured on Plate III



I. NORMAL RABBIT SERUM + BULLOCK'S ERYTHROCYTES.
Control Series

Showing that normal rabbit serum exerts no haemolytic effect upon bullock's erythrocytes.

The circular spots are dark in the centre and show a broad clear margin throughout the series. The dark central area is due to intact erythrocytes which have not spread as far as the fluid in which they are suspended. The clear colourless zone shows that the normal serum dilution has not caused haemolysis, for if it had (see first numbers of next series), the whole area of diffusion of the fluid containing haemoglobin in solution would appear dark.



II. IMMUNE RABBIT SERUM + BULLOCK'S ERYTHROCYTES.

Showing that the serum of a rabbit immunised against bullock's erythrocytes exerts a haemolytic and agglutinating action on these erythrocytes.

The circular areas are uniformly dark where complete haemolysis has occurred. Where agglutination of the erythrocytes has taken place, they diffuse irregularly. Towards the end of the series the diffusion approximates to that in the series above.



III. HEATED IMMUNE SERUM + BULLOCK'S ERYTHROCYTES.

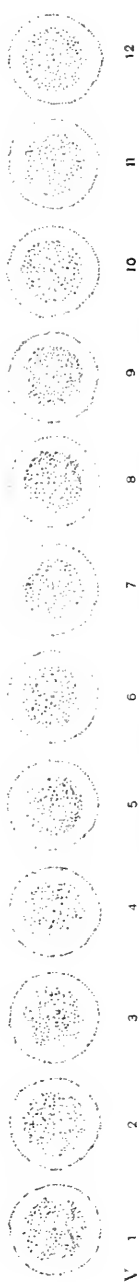
Shows that the haemolytic power has been abolished, but agglutinative power retained, and therefore proving that the two phenomena are independent, and that rapid haemolysis may mask agglutination as in series II above.



IV. IMMUNE SERUM OF A DIFFERENT RABBIT + BULLOCK'S ERYTHROCYTES.

Showing that haemolytic power may be acquired in the absence of agglutination.

The gradual diminution in the darkness of the area of diffusion is accompanied by an equally gradual appearance of erythrocytes near the centre, and ultimately the two zones characteristic of the control series above appear with the disappearance of haemolysis.



V. THE SAME IMMUNE SERUM AFTER HEATING + BULLOCK'S ERYTHROCYTES.

Showing that haemolysis had not masked agglutination in series IV and that haemolytic but not agglutinative power had been acquired. The serum appears to be as indifferent as that in the control series I above.

PLATE IV. *Graphic record of the experimental analysis of the fundamental phenomena of Haemolysis represented on Plates II. and III.*

Determination of Constants*.
























[illegible]

Analysis of the part played by the two haemolytic factors.

+ ♀	○
+ ♀	○
+ ♂	○
+ ♂	○
+ ♂	○
+ ♂	○

VII. Centrifuged fluid. Haemolytic action of the fluid which had contained 0.25 c.c. normal serum, when fresh erythrocytes and 0.0035 c.c. heated immune serum are added.

VIII. Centrifuged erythrocytes. Inactivity of heated immune serum on bullock's erythrocytes after the latter have been in contact with 0.95 c.c. of normal serum.

 	
 	 
 	 $+$ 
 	 $+$ 
 	 $+$ 
 	 $+$ 

V. Centrifuged fluid. Inactivity of the fluid which had contained a haemolytic dose of immune serum before contact with bullock's erythrocytes.

TI. Centrifugalised erythrocytes.
Haemolytic action of normal serum on bullock's erythrocytes after the latter have been in contact with a haemolytic dose of heated immune serum.

PLATE V. *Graphic record of the unaugmented haemolytic action of the serum of a rabbit immunised first by subcutaneous injection of bullock's erythrocytes and 24 days later rendered more highly immune by intraperitoneal injection of erythrocytes saturated with heated immune serum.*

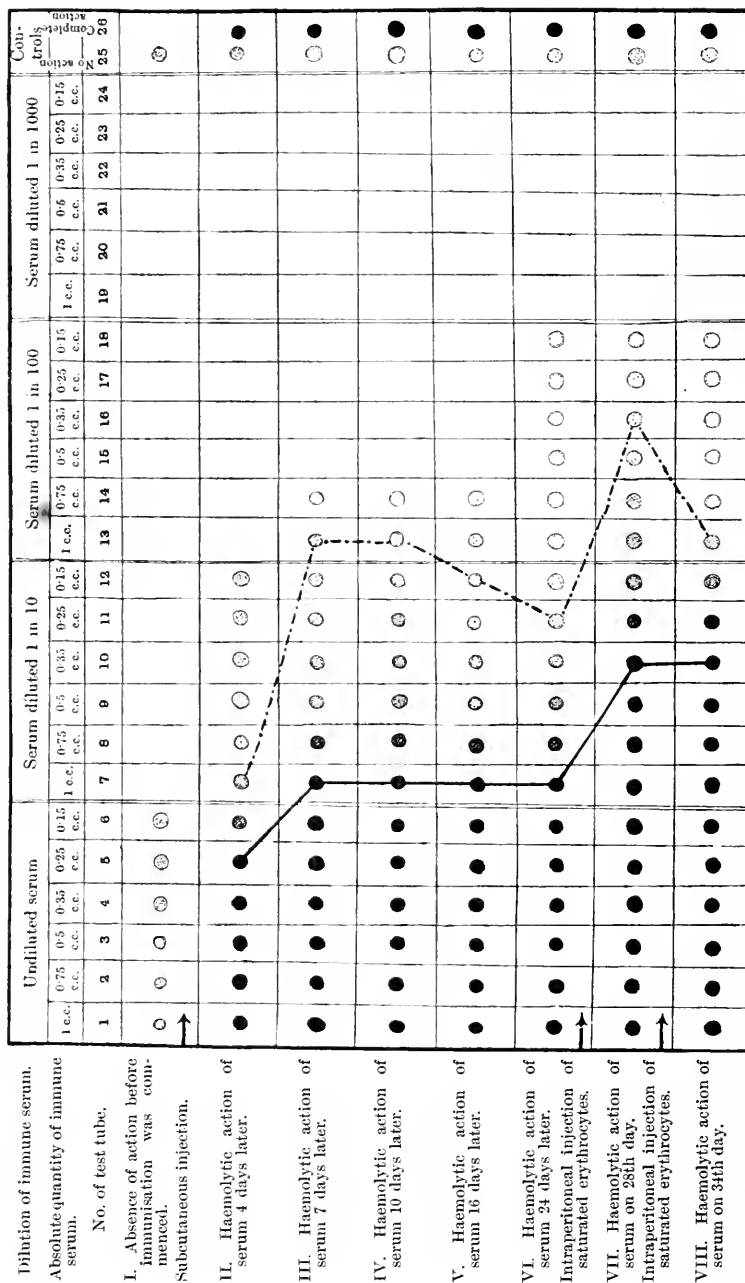
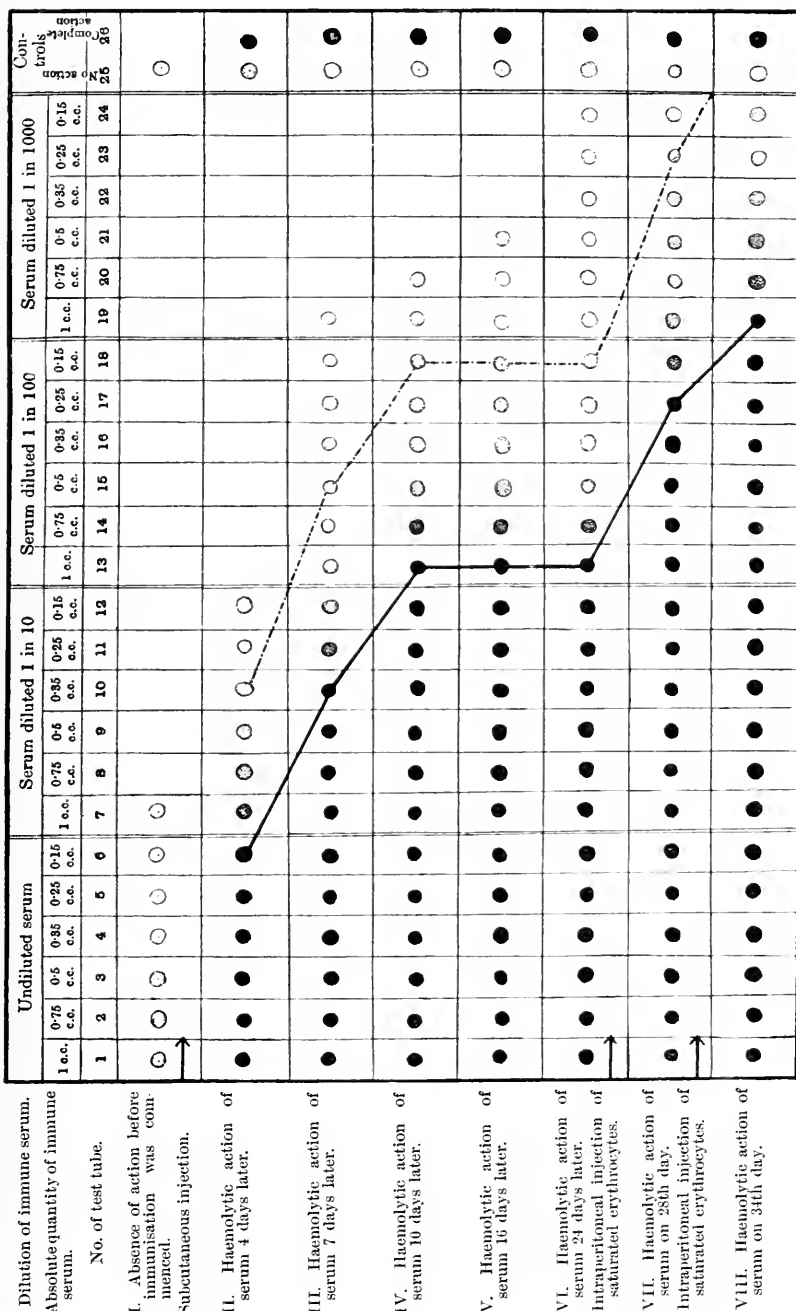
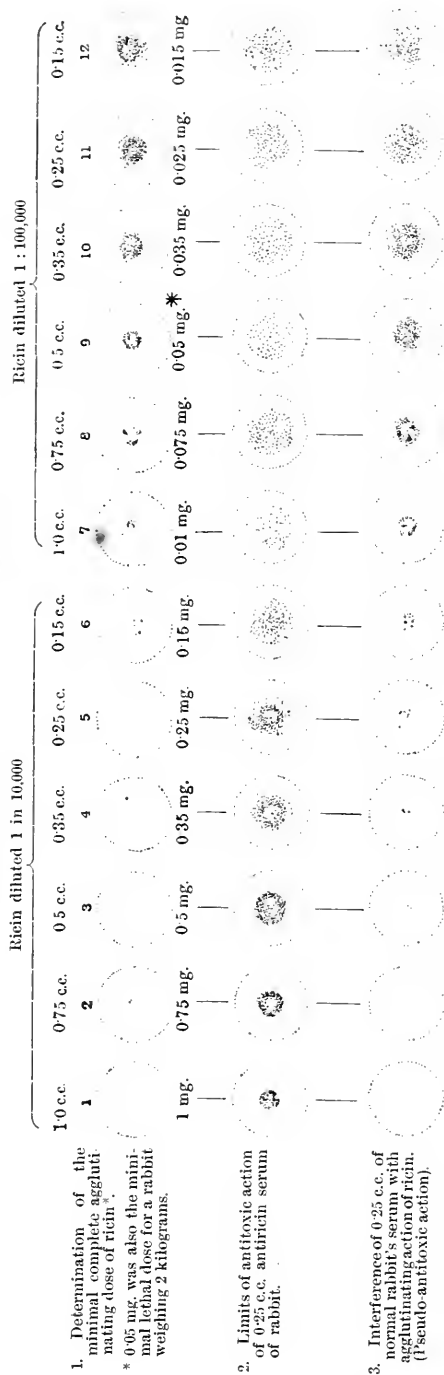


PLATE VI. Graphic record of the augmented haemolytic action of the same rabbit's serum as on Pl. V. In this series of experiments the haemolytic action of the heated immune serum has been augmented by the addition of a normal rabbit's serum (0.25 c.c.).
The agglutinative action no longer interferes with the limits of haemolysis.



— The continuous line marks the limit of complete haemolysis.
 • • • • • The dotted line passes through the tubes in which a trace of haemolysis was still present.
 The apparent retrocession of this limit is due to agglutination.

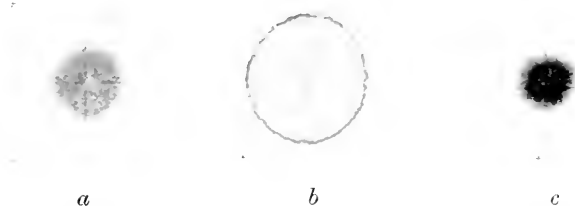
PLATE VII. *Erythrocytes as indicators of the presence of free ricin.*

Exp. 4, 5 and 6. Ricin and antiricin present in constant quantities throughout. (Ricin 0.05 mg. Antiricin 0.05 c.c.)

4. Antagonistic action of the serum of a passively immunised guinea-pig to the antiricin function of the serum of a rabbit actively immune.
5. Absence of this antagonistic action in the case of a normal guinea-pig.
6. Consequences of mere dilution. Normal saline replaces guinea-pig serum in experiments 4 and 5.

Control. Saline replaces ricin. The combined sera do not produce clumping.

Control. Normal saline only. No action.



DIFFUSION ON BLOTting PAPER OF ERYTHROCYTES AND THEIR HAEMOGLOBIN CONTENTS.

- (a) Erythrocytes suspended in an indifferent (non-haemolytic) serum.
- (b) In a serum causing complete haemolysis.
- (c) In a serum causing agglutination.

(Photograph. natural size).



“ANKYLOSTOMIASIS.” No. II.

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IN a previous paper (Volume III., p. 95 of this *Journal*) we gave an account of our observations on cases of ankylostomiasis in Cornish mines. The present paper will be devoted to a more general discussion of the disease from the point of view of its prevention, particularly in mines.

Distribution of Ankylostomiasis.

Ankylostomiasis is known to exist as an endemic disease in warm countries all over the world. The countries or districts within which it prevails to a serious extent appear to lie within about 35° north or south of the equator; but persons more or less infected with the worm, and occasional cases of the disease itself, are, in Europe at least, found among the agricultural population even north of latitude 45°, in Northern Italy, where the *Ankylostoma duodenale* was originally discovered, and in Hungary, where it has quite recently been found¹. There are still, however, many countries or districts as to

¹ Iberer, *Münchener medizinische Wochenschrift*, June 9, 1903.

which there is no definite information with regard to the presence or absence of a more or less widespread infection with the worm. It seems probable, for instance, that it may exist over the Spanish peninsula, and possibly in the south of France, as well as in the European countries to the south of Austria. It is certainly a very remarkable fact with regard to ankylostomiasis that though it has been known for 50 years, and its recognition is a matter of great practical importance, it is nevertheless so frequently not recognised. This was the case with the Cornish outbreak, for instance; and a still more striking instance is that of its prevalence among the agricultural population in many parts of the Southern States of America, from North Carolina to Texas. Although Hirsch¹ pointed out more than 20 years ago that judging from the descriptions in medical literature ankylostomiasis is common in the Southern States, yet it appears that the disease remained unrecognised locally, and consequently untreated, till about a year ago, when its identity and wide prevalence were established by Stiles².

In the course of his investigation of ankylostomiasis in the United States, Stiles discovered that the worm commonly found there is of a different species from that described in European cases. He also found that worms from cases in Porto Rico and Cuba were of the American species. It thus appears probable that the ankylostomiasis which is so prevalent in British and other West Indian Islands, and possibly that in Brazil and other South American countries, may be partly at least due to the American species. Schneider identified worms obtained by Wucherer in Brazil about 1866 as *A. duodenale*, and Capps³ has recently observed the same species in a case from Panama, but Pieri

¹ *Handbuch der historisch-geographischen Pathologie*, 1881.

² "Report upon the Prevalence and Distribution of Hook-worm Disease in the United States." *Bulletin No. 10, Hyg. Lab. U.S. Pub. Health and Mar. Hosp. Service*, 1903. The American species has only one median tooth, in place of the two dorsal teeth and four ventral hooks which surround the mouth of the *A. duodenale*. The bursa of the male worm is also different in the two species, and the eggs of the American worm measure, according to Stiles, about 64 to 76 μ by 36 to 40 μ instead of a mean of about 59 by 37 μ as in the case of the Old World species. Stiles adopts the names *Uncinaria duodenalis* and *Uncinaria americana* for the two worms, and calls the disease Uncinariasis or Hook-worm disease. The zoological grounds for this change of nomenclature, which is certainly very confusing, are criticised by Looss (*Centralblatt f. Bakt.* xxxi. p. 422, 1902). The American species has no hooks of any kind, and the disease has been generally known as ankylostomiasis or anchylostomiasis for 25 years.

³ *Journ. of American Med. Assoc.* Vol. XL. p. 28, 1903.

has observed *A. americanum* in a case from Brazil¹. Von Linstow² reports that he has found the same species in the intestine of the West African Chimpanzee, and suggests that the American infection has originated from slaves brought over from Africa. It does not seem to be definitely ascertained as yet to which species the ankylostomiasis met with in various parts of Africa outside Egypt, and elsewhere throughout the world, is due. *A. duodenale* was identified by Stiles in a case from the Philippines. The worms examined by us in Cornwall were *A. duodenale*.

In warm countries ankylostomiasis occurs among the agricultural population, and seems to be nearly always associated with gross faecal pollution of the soil round dwellings. The disease does not originate in towns, where there are always arrangements of some kind for the disposal of excreta; but it has been repeatedly observed among men employed in brick-making, even as far north as Cologne. Faecal pollution of the wet clay in the clay pits during warm summer weather doubtless accounts for the infection. By far the most serious trouble from ankylostomiasis in temperate climates has, however, arisen in connection with deep mines and tunnels under mountains; and we propose, therefore, to devote special attention to this subject.

Serious attention was first drawn to ankylostomiasis in Europe by the discovery that a great outbreak of anaemia among the men engaged in making the St Gothard Tunnel was in reality ankylostomiasis. The worms were first discovered at post-mortem examinations on tunnel workers who had died in hospital. Owing, however, to the prevalent belief in "miners' anaemia" due to bad air or other causes it was some time before, through the work chiefly of Perroncito³, proofs were afforded that the tunnel disease was simply ankylostomiasis, and that it was easily curable by large doses of certain vermifuge remedies, of which the most suitable were found to be extract of male fern and thymol. Perroncito found that the same disease existed among miners at Kremnitz in Hungary, and it was soon afterwards found among colliers at St Étienne in France, Liège in Belgium, Brennberg in Hungary, Aachen and Westphalia in Germany. Recently the disease has spread greatly in Belgian and German collieries, and in some of the Hungarian collieries it has also been extremely troublesome.

From old medical records it appears probable that ankylostomiasis existed in many European mines previously to the St Gothard

¹ *Centralbl. f. Bakteriöl.* Vol. xxxiv. p. 533, 1903.

² *Centralbl. f. Bakteriöl.* (1) Vol. xxxiv. p. 527, 1903.

³ See Perroncito, *Archives Italiennes de Biologie*, Vol. II. p. 315 (1882) and III. p. 7.

outbreak. It is difficult, for instance, to conclude that the epidemic of anaemia at Anzin near the Belgian frontier of France about 1802 was anything else but ankylostomiasis. "Brickmakers' Anaemia," which was definitely shown in 1882 to be ankylostomiasis, is known to have existed near Cologne since 1872 and near Bonn since 1878. The anaemia in Hungarian mines appears also to have existed before the St Gothard Tunnel was made. Undoubtedly, the dispersal over Europe of the St Gothard workers must have spread the disease, and the Belgian collieries may well have been infected from this source. Owing, however, to the readiness with which the existence of ankylostomiasis escapes diagnosis it is very difficult to trace definitely the sources of infection. About 1893 cases began to be noticed by Loebker at the deep collieries of the great Westphalian Colliery district. The infection was probably brought by miners from Hungary. About the same time a number of cases began to be noticed at deep Belgian coal-mines. The numbers of cases of definite anaemia observed at Westphalian collieries by the Medical Officers of the Knappschafts-Verein (a compulsory insurance association for miners) were as follows since 1896:

1896	107	1900	275
1897	113	1901	1030
1898	99	1902	1355
1899	94				

The great increase in the number of cases, from 1900, was coincident with the introduction of a compulsory system of watering every working part of the collieries in order to prevent coal-dust explosions. In the Belgian deep collieries the disease has spread greatly during the last ten years, though definite figures as to the number of cases of illness are not available. The French collieries seem to have suffered very slightly. As already mentioned in our previous paper, the recent outbreak of ankylostomiasis in Cornish mines seems to have begun about 1894, though it was not recognised till the end of 1902. Judging from statements made to us by old miners, however, it seems possible that there may have been a previous outbreak at Dolcoath Mine. Dr J. B. Montgomery of Penzance has communicated to us the interesting fact that about 50 years ago he observed a group of cases of persistent anaemia among miners who had returned from Chili, and that their symptoms seemed to correspond to ankylostomiasis. Dr Jago of St Just has also observed similar cases much more recently among returned miners. It thus seems not improbable that the infection may

have been brought to Cornish mines long ago. As many Cornish miners have gone to collieries in various parts of England they have presumably carried the infection with them. Infection may also have been easily brought to England by Italian or other foreign miners, or by men returning from tropical countries. Thus a case of ankylostomiasis in a collier was recently recorded by Stockman¹. The patient had recently returned from military service in India, and was sent from a Scotch colliery to the Glasgow Royal Infirmary, where he was found to be suffering from ankylostomiasis. As yet, however, no outbreak of ankylostomiasis has been discovered in any colliery in Great Britain. In the collieries of the United States and Canada there is likewise no recorded outbreak, though Stiles records cases among miners in the infected Southern States. Many Hungarians are reported to be employed in coal-mines in the United States.

It may be taken for granted that ankylostomiasis exists among the miners in nearly all tropical and warm countries; but information on the subject is very scanty. Cases have been recorded at the Kimberley diamond mines: also in Queensland, so that the disease may perhaps exist in Australian mines. We ourselves observed that a white miner just returned from Mysore was infected. The disease has not been observed in the gold-mines of the Transvaal, although presumably hundreds of infected natives are employed in the mines. Possibly the Transvaal mines, which are very dry, do not permit of its spread. It would, however, be rash to assume that ankylostomiasis is absent in mines in any country, unless it has been definitely looked for and found to be absent.

Life-History of the Ankylostoma.

The successful prevention of the spread of ankylostomiasis depends mainly on a knowledge of the conditions under which the *Ankylostoma* propagates itself. We propose therefore to discuss the life-history of the worm from this point of view.

The records of post-mortem examinations show that the adult worms, male and female, live in the upper part of the small intestine, mostly about the upper part of the jejunum. During life they are probably nearly always firmly adherent to the mucous membrane, though they can apparently change their positions.

¹ *Brit. Med. Journ.* Vol. II. p. 189, 1903.

The adult worms may live in the human bowel for several years. This is a point which it was almost impossible to settle satisfactorily in regions such as Egypt and India where there is an infection of the general population; but in Cornwall, where the miners are alone infected, and in the United States, where infection is confined to the Southern States, several clear cases lasting for about five years have been recorded. C. W. Stiles¹ has found cases in children who were inmates of an institution (where reinfection seems to have been satisfactorily excluded) to which they had been removed from an infected area between six and seven years previously. In Cornwall we met with a youth (R. S., case xvi.²) who gave up underground work in consequence of anaemic symptoms which developed shortly after he began working in Dolcoath. Rather more than four years afterwards we found that his stools still contained a few eggs. During this time he had been engaged in driving a butcher's cart and had never even been to a mine. In this case there does not seem to have been the slightest chance that he could have become infected in any way since leaving Dolcoath. In another case a miner from Dolcoath (case xxxviii. p. 133) had been confined to his home (six miles from the mine) by miners' phthisis, and 20 months after his last contact with the mine we obtained numerous worms after thymol treatment. In Westphalia it has been repeatedly observed that miners returning from two years' military service are still infected. The butcher's boy whom we have mentioned had entirely recovered from his anaemia when we saw him (Hb 99 %), and he was to all appearances in good health. Such cases illustrate the insidious way in which *Ankylostoma* might gain entry to a mine, and the dangerous character of apparently harmless individuals.

Though it is thus clear that the worm may live a long time, we have evidence that in many cases they soon die out. C. F. Fearnside³ examined prisoners when they were first admitted to an Indian gaol, and also those who had been inmates for more than six months. With proper sanitary arrangements, it is extremely unlikely that anyone would become infected while under detention. He found that 68 % of the fresh cases were infected with *Ankylostoma*, but only 58 % of the older inmates. In the U.S. Government Hospital for the Insane it has been found by P. E. Garrison and others that the frequency of infection with all intestinal parasites diminishes with the length of residence⁴.

¹ *Loc. cit.* p. 58.

² *Journal of Hygiene*, Vol. III. p. 130.

³ *Brit. Med. Journ.* 1900, II. p. 541.

⁴ *Treasury Dept. U.S. Hygiene Lab. Bulletin* 13, 1903.

In Cornwall we met with at least one case in whom the worms appear to have completely died out without any specific treatment (J. W., case xxii., Vol. III. p. 131). He had suffered severely from pallor and dyspnoea six years before and had in consequence given up underground work for employment on the surface in a machine shop. His faeces contained no eggs and have been examined on several occasions.

It seems then that the adult worms are capable of such longevity that one can only be sure that an individual, who in the past has had contact with an infected place, is not infected by showing that his stools are free from eggs. For this purpose it is necessary that the examinations should be made very thoroughly and on more than one occasion. No importance at all should be attached to the fact that such a case has recovered from symptoms of anaemia. We have seen numerous cases of complete or partial recovery subsequent to an exchange of underground for surface employment, but eggs were found in nearly every instance.

It is thus quite evident that a person who has once been even slightly infected may, if the conditions be favourable, as in a warm and damp mine, spread the disease many years after he has himself acquired the infection—perhaps in some other country.

Although the *Ankylostoma* ova must often remain for several days in the intestine they are apparently never very far advanced in development when passed. In fresh or nearly fresh faeces we have usually found the eggs at the 4-cell to 16-cell stages. The 2-cell stage is not infrequently seen, but we have never observed unsegmented ova, or ova which had passed the morula stage. They will hatch in a few hours if the faeces are kept moist and warm, or in a few days at the temperature of a fairly warm room. All authorities are agreed that the eggs require free oxygen for their development. The reason why they do not develop further in the intestine is presumably, therefore, that free oxygen is lacking. If the adult *Ankylostoma* is also, unlike some other intestinal worms, aerobic, we can better understand why it fixes itself to the intestinal wall, since it will there obtain free oxygen as well as nutriment.

The ova appear to be as a rule pretty evenly distributed in a given sample of faeces; but it would seem that they may be absent for a short time although living worms are still present in the intestine. It has been repeatedly stated, for instance, that when ova have disappeared from the faeces shortly after the administration of extract of male fern or thymol, they have been found again a few days later. In

any case it seems hardly probable that any very definite relationship can exist between the number of ova present in a given weight of faeces and the number of worms in the intestine.

When the faeces are exposed to air outside the body, and kept from drying, the ova will develop and hatch within a wide range of temperature. After the cell-mass in the ovum has reached the morula stage it bends round within the shell, assuming a comma form. As the body elongates further it begins to show very active writhing movements, and finally assumes a figure of eight form, when the movements cease. This period of quiescence seems always, so far as we have been able to observe, to precede rupture of the shell and emergence of the larva.

The accurate investigation of the conditions under which eggs and larvae develop is a matter of no small difficulty. Artificial cultivations seldom seem to realise the optimum conditions for the development of the eggs and the growth of the larvae, and one lot of faeces will give very different results from those obtained from another. In some faeces the eggs hatch out quickly and the larvae live and grow well, while in another the young larvae die as soon as they are hatched. In some samples even we have failed to get the eggs to hatch at all under precisely the same conditions which were favourable in other cases. Possibly these ova were not impregnated, but we can offer no certain explanation of the differences which we have observed. They do not seem to depend on variations in the reaction of the faeces (which has always been acid in samples more than a day or two old). In several instances we have noticed that the eggs will not hatch or only hatch very slowly in fluid stools, while in more solid samples from the same individual they have grown well. On the other hand, variations almost as great have been noted in different samples of about the same consistency from the same patient.

Under the circumstances we propose to give a short account of the more important facts which have been ascertained in this connection without attempting to traverse the whole of the extensive literature on the subject, and with reservations which the apparently inconsequential irregularity of the experimental results renders necessary.

A. Temperature. The eggs are killed in about 24 hours by exposure to a temperature of about 40° C. (104° F.) or to a nearly freezing temperature. They will not hatch in 6 weeks under the variations of outdoor temperature found in London in November and December (0·5° to 12·5° C., 33° to 54° F.), nor in 5 weeks at the

temperature of the laboratory at Dolcoath in November, where the thermometer during the day-time generally rose to between 11° and 13° C. (52° to 55° F.). The eggs may hatch at any temperature from 16° C. (61° F.) to 38° C. (100° F.). In an ordinary hot incubator (37° C. = 98·5° F.) the eggs often die before hatching, but a few larvae sometimes hatch out in about 24 hours and then rapidly die away, seldom reaching the "encapsuled" stage.

In a series of cultures watched for five days we found that at a temperature of about 32° C. some of the ova were hatched within 24 hours. At 37° development was less rapid, and usually arrested before the stage of hatching. Even at 32°, in one experiment, development was completely arrested just before the hatching stage, and no further changes were observed after the first 24 hours. At 30° we observed a retardation of hatching in most of the ova, though development up to the hatching stage was about as rapid as at 32°, and after three days about two-thirds of the eggs were hatched. At 27° most of the eggs were hatched within 2 days, and nearly all in 3 days, but none within 1 day. At 24° only about a third had hatched after 2 days, but nearly all in 3 days. At 21° none had hatched in 2 days, about half in 3 days, and nearly all in 4 days. At 18° about a fifth were hatched after 3 days, and nearly all after 4 days. At 16° about a fifth were hatched after 4 days, and nearly all after 5 days. At 13° none were hatched after 5 days, but about a third seemed just ready to hatch. In this series of cultures, which were in covered Petri-dishes on moist sand, none of the free larvae continued to grow, possibly because the faeces were too dry. It will be seen, however, that at temperatures from 30° to 16° nearly all the eggs hatched within 5 days.

After hatching, the larvae, under suitable conditions, continue to grow, and after moulting once at least, reach a stage at which they are about 0·6 mm. in length and actively motile. The chitinous sheath is now very prominent, and can usually be seen to be more or less separated from the contained larva, the head and tail of which are often retracted from the ends of the sheath. There are no sexual differences, or signs of any mode of reproduction, and outside the body the larvae never pass beyond this the so-called "encapsuled" stage which, as will be seen below, is the infective one¹.

¹ The further stages of development within the body have been investigated by Looss, *Centralbl. f. Bakt.* (1), 1897, p. 913. A short and copiously illustrated summary of what is known as to the structure and stages of development of *Ankylostoma* is given by Stiles, *loc. cit.*

We have observed these full-grown larvae in samples of faeces left in bottles at the ordinary room temperature of about 15° or 16°. They appear, however, to develop much more readily when the faeces are placed in a covered dish with a very shallow layer of water in the bottom, and they are found swimming about in enormous numbers in the water.

Practically it is of course very important to find the minimum temperature at which "encapsuled" larvae can be grown: the limit has been placed at 20° C. (68° F.) by Bruns, who has studied the matter very carefully¹. This is far above the mean annual temperature of any part of England and also higher than the temperature in many mines. We have however on two occasions and with two different lots of faeces succeeded in hatching the eggs and growing the larvae to the infective stage at a temperature which at no time exceeded 17° C. (62·5°) and was nearly always 15·9° C. (60·6°). The larvae seemed to be very vigorous, as did also a culture grown at 19° (66°). The critical point seems then to lie somewhere between 13° and 16° C. (55° and 60° F.). Below this temperature the eggs will not hatch, while above it they may do so and the larvae may grow to the "encapsuled" stage. It is not however to be assumed that all samples of infected faeces will produce larvae at 17° C.: the optimum temperature seems to be about 24° C. (75° F.). Samples of infected faeces placed in jars in Dolcoath Mine (temp. 25° C.=77° F.) showed numerous larvae at the end of five and seven days, but none in 48 hours.

We have hardly any information as to the influence of variations of temperature on the development of the eggs. This is the condition under which eggs would be in the open air in England, and it would be of importance in cases where the daily maximum is above, while the minimum is below, the critical limit which we have endeavoured to define. There seems to be no reason why the eggs should not hatch at 60° F., a temperature which is often found in the open air in this country. We are, however, ignorant of the influence which a daily reduction of temperature to below 60° may have. The daily minimum in England is rarely more than 60°². The time during which they are

¹ Quoted by Haldane, Report to the Home Secretary on Ankylostomiasis in Westphalian Collieries, *Parliamentary Paper* [Cd. 1843], 1903.

² The following data (extracted from the Greenwich Records for the fifty years 1841 to 1890) may be of interest. Mean daily temperature more than 15·5° C. (60° F.) on 71, and less than 4·4° C. (40° F.) on 56 days per annum. The mean maximum temperature is more than 15·5° C. (60° F.) on 162 days, and more than 21° C. (70° F.) on 86 days, while the mean minimum is below 1·7° C. (35° F.) on 87 days. The average number of days per

exposed to the necessary warmth is also no doubt of great importance: in our laboratory experiments four to eight days were taken to hatch the eggs at 16° C. (61° F.), and another three or four weeks for the larvae to reach the "encapsuled" stage. This time is necessarily prolonged if at intervals the culture is exposed to lower temperatures. It cannot however be denied that there is a possibility that eggs left in the open in England might hatch and the larvae grow to an infective stage during the hotter months of a favourable year (June to August). In almost every part of this country however the spread of *Ankylostoma* is practically checked by the proper use of sanitary arrangements, and it is extremely improbable that any eggs would come to maturity in face of the temperature combined with systematic sewage disposal.

As far as we know the spread and prevalence of the disease depends on the development of the larvae to the infective stage, and there is no better illustration extant of the effects of temperature than the following table, compiled from figures quoted by Dr Tenholt with regard to collieries in Westphalia¹.

Temperatures about working face	Number of collieries 1901	Number of men employed underground 1901	Cases of anaemia per 1000 men employed underground
Below 17° (63° F.)	67	36033	0·6
17° to 20° (63° to 68° F.)	84	68604	0·4
20° to 22° (68° to 72° F.)	45	43710	2·5
22° to 25° (72° to 77° F.)	33	39836	11·7
Over 25° (77° F.)	12	9853	39·9

Dr Tenholt further found that the cases found in the mines with a temperature of less than about 22° C. appeared to be among men who had come from mines with a higher temperature, where they were doubtless infected.

annum on which the temperature rises above 21° C. (70° F.) is 77, and on nearly 4 days per annum does the minimum fail to fall below 15·5° C. (60° F.). The average number of days on which the temperature falls below freezing-point is 56, on nearly 5 of which it remains continually below 0° for 24 hours.

These figures must not be applied too closely to the *Ankylostoma* problem. Other factors have to be taken into consideration, such as exposure to direct sunshine in sheltered places such as quarries, cooling of the faecal mass by evaporation, &c. If, as appears to be the case, small variations of temperature about 16° C. are important, different parts of England must be considered separately. The mean annual temperature at Greenwich for the years 1841—1890 was 9·7° C. (49·46° F.), while that of Penzance is 11·5° C. (52° F.). These figures are misleading in the sense that they tell us nothing of the prevalence of hot spells in the summer and of spells of cold in the winter of sufficient severity to kill *Ankylostoma* eggs and larvae.

¹ See Haldane, *loc. cit.*

B. Moisture. All observers agree that thorough drying will kill both eggs and young and old larvae, and with this our own experience agrees. On the other hand, immersion in water prevents the eggs from developing and in a few days kills them. The larvae on the contrary do well in water. The degree of drying necessary to kill the eggs and larvae is a high one, and is only attained slowly in the interior of a large mass of faeces, but in many instances probably suffices to render deposits harmless. The great importance of adequate moisture is shown by the history of the Westphalian collieries, where the introduction of compulsory watering of the roads to prevent dust explosions in dry collieries was at once followed by the blazing up of what had previously been but a smouldering infection.

C. Oxygen. A fairly free supply of oxygen is necessary for the development of the eggs and for the growth of the larvae. This is shown by the absolute failure of eggs to develop at any temperature when sealed up, or even when covered with a thick layer of water. Absence of oxygen is not quickly fatal however: in one of our experiments eggs were sealed up and kept at about 30° for nine days. At the end of this period none had advanced at all, but 48 hours after air had been freely admitted to the culture nearly all the eggs contained young embryos. Lambinet estimates that it takes about 10 days' immersion in water to *kill* the eggs.

D. Light. It has been found by several observers that exposure to direct or even diffuse sunlight is sufficient to prevent development and to kill within about 48 hours.

E. Disinfectants. The eggshell of *Ankylostoma* is probably composed of chitin, an insoluble and impenetrable substance which also forms the sheath of the "encapsuled" larvae¹. It is presumably the possession of this coat which confers on them the high powers of resistance which they exhibit towards the ordinary disinfectants, etc. Perroncito² observed this many years ago, and it has been constantly confirmed by other writers. Thus J. Lambinet³ found that immersion of the eggs in such powerful solutions as 2 p.c. corrosive sublimate or 3 p.c. lysol for one hour will not prevent their subsequent development when removed to a medium free from the reagent: while glycerine or strong salt solution rapidly destroys the eggs, acting of course as dehydrating

¹ This has never, so far as we are aware, been definitely shown for *Ankylostoma*, but a chitinous coating is certainly present in most Nematodes.

² *Arch. Ital. de Biol.* III. 1882, p. 7.

³ *Bull. Acad. Roy. de Médecine de Belgique* (4) xv. 1901, p. 397.

agents. The same observer found that in one hour eau de Labarraque or eau de Javelle (sodium and potassium hypochlorite) will not effectively penetrate the shell: this is of considerable interest since, with the exception of strong mineral acids, hypochlorite will alone dissolve chitin. We have found that hot solutions of eau de Javelle dissolve the egg-shell of *Ankylostoma* in a few minutes; the action of these solutions on chitin in the cold does not advance beyond softening in a reasonable time.

When the action of reagents upon the larvae is considered, it is necessary to distinguish carefully the stage of growth of the larvae in question. On first emerging from the egg they have no special chitinous coat and are very easily killed by 0.1 p.c. sublimate or any other germicide. It is a common observation that at high temperatures (37° C.) the eggs often hatch very quickly, but that the young larvae die almost immediately. It is very possible in these cases that they are killed by some substances in the decomposing faeces. When the larvae have become "encapsuled," they show great resistance. Thus Lambinet found that 2 p.c. sublimate does not kill in six hours, while in 25 p.c. sulphuric acid they live for $\frac{3}{4}$ hour. Other observers have obtained similar, though perhaps not such exaggerated, results. We have noticed ourselves that large larvae will live for more than half-an-hour in 2½ p.c. sublimate in 1 p.c. acetic acid.

But when viewed as a practical question, the matter assumes rather a different light. It is quite true that both eggs and large larvae will withstand for a time very powerfully toxic reagents, and will develop and live when removed from such solutions to blander surroundings. In practice however disinfectants are applied to stools and allowed to remain in contact with them for a considerable time: it is then of no moment if the eggs do develop since the newly-hatched larvae will soon be killed by the disinfectant¹. Even when "encapsuled" larvae are concerned, a moderate extension of the time limit during which they are exposed to the solution will experimentally give results which encourage the practical use of disinfectants. We have found, and have confirmed the experiments several times, that comparatively

¹ This is true of course only with some dry system of dealing with faeces. With a water-borne system there is no danger from infected stools except possibly on sewage farms. In some places underground it is true that the water will wash any disinfectant away from the faeces: but under such circumstances the abundance of water will of itself be a great hindrance to the development of the eggs.

weak solutions will in about 24 hours destroy vigorous "encapsuled" larvae. The following data were obtained from a culture six weeks old; a small number of larvae were immersed in about $\frac{1}{2}$ in. depth of the solutions:—

	16 hours	48 hours	36 days
London tap-water	lively	lively	lively
+ 1·8 p.c. NaCl	lively	lively	lively
+ 0·2 p.c. corrosive sublimate	dead		
+ 0·1 p.c. corrosive sublimate	dead		
+ 1 p.c. izarl	dead		
+ 0·4 p.c. izarl	most dead	all dead	
+ 0·2 p.c. izarl	most dead	all dead	

It is probable that a shorter time is sufficient, but one cannot accurately judge of the death of these large larvae until structural changes are perceptible. Cessation of movement (which occurs after about 1 hour in 0·1 p.c. sublimate) does not necessarily indicate death. With the exception of plain tap-water, the solutions mentioned were very soon fatal to young larvae.

In the same way the eggs are killed if the disinfectant is allowed to remain in contact with them for some time. A few drops of formalin, for instance, added to a two ounce bottle half full of faeces, will effectually prevent the development of the eggs.

Longevity of Eggs and Larvae. The question of how long eggs and larvae will live without coming into relation with human beings, is one of great practical importance, and it is to be regretted that the information on these points which is at our disposal is far from complete.

If faeces containing ova are left in the open air during an average English autumn with no frost, drying being prevented, many soon die, but a fair number remain capable of developing into larvae at a suitable temperature for at least six weeks, and at the end of that time there is no indication that the number of live eggs is materially diminishing, though their capacity to hatch quickly is seriously diminished. Frost kills the eggs, but apart from this there seems to be no reason for supposing that infected faeces will become innocuous if left alone for several months.

Our information with regard to the longevity of the larvae is little more precise. In some cultures the larvae die off rapidly as soon as they are hatched: in others they grow to the so-called "encapsuled" stage and then die quickly. In other instances again they will live for months

at this stage with a very small mortality. Dr Bruns in Westphalia has thus maintained cultures actively virulent for at least six months¹, and Leichtenstern for seven months. The longevity of the larvae seems then to be considerable under favourable circumstances; but it also depends to a large extent on small details of the surroundings (nature of faeces, temperature, moisture, etc.), which are at present not at all fully understood.

It is not improbable that conditions in the soil, apart from moisture and temperature, influence the development of the larvae. Stiles reports, for instance, that ankylostomiasis in the Southern States of America is chiefly confined to the dwellers on sandy soil, and that those living on clay are almost immune.

Infective Stage. Although it was pointed out by Grassi and Parona in 1879 that the infective stage of the *Ankylostoma* is probably the developed larval worm, definite experimental evidence of this fact was first furnished by Leichtenstern². The latter observer showed that if either the ova or the freshly hatched larvae are swallowed by men, the adult worm does not develop in the intestine, since no ova appear in the faeces. On the other hand, if the fully developed, "encapsuled" larvae be swallowed, ova begin to appear in the faeces after about a month. The latter observation has recently been confirmed by Pieri³, with a culture of larvae from the American variety of *Ankylostoma*, the ova appearing in the faeces after 30 days.

Paths of Infection. It is quite evident from these experiments that one source of infection by *Ankylostoma* is through the mouth; and till recently it was assumed that this is the sole source of infection. In 1898 Looss⁴ made the observation that after a few drops of a culture of ripe *Ankylostoma* larvae had accidentally dropped on his hand, the skin at the place became inflamed; and some time afterwards he found that he was suffering from ankylostomiasis. As it appeared to him very improbable that he had been infected through the mouth, and as the disease is not endemic in Cairo, where he was working, he concluded that he had probably been infected through the skin. Some time later he made the further experiment of smearing a few drops of a culture on the skin of the leg of a hospital patient shortly before the leg had to be amputated. On excising the skin and cutting sections he

¹ Haldane, *loc. cit.* p. 5.

² *Centralbl. f. klin. Med.* 1886, p. 675.

³ *Centralbl. f. Bakteriologie*. Vol. 34, p. 533, 1903.

⁴ *Centralbl. f. Bakteriologie*. Vol. 24, p. 486, 1898; Vol. 29, p. 733, 1901.

found larvae in the subcutaneous tissue, into which they had apparently penetrated through the hair follicles. He also found that the larvae disappear very rapidly when placed on the skin, and that after a short time only empty sheaths are to be found, while a localised dermatitis is quickly produced. The disappearance of the larvae, and dermatitis, have also been recently observed by Pieri¹ in two experiments. In 1902 Bentley², who had seen the first paper by Looss, observing that the skin-disease known in Assam as "pani-ghao," "water-itch," or "ground-itch," was associated with the prevalence of ankylostomiasis, made a series of experiments on the subject. Water-itch occurs in the wet season, and affects the feet of coolies who work barefooted in polluted earth. A few hours after exposure of the feet to the polluted ground intense itching and burning at the affected place is felt. A vesicular eruption follows in about a day, and the vesicles commonly become pustules. In bad cases the skin may slough. The disease appears also to exist in the sugar plantations of the West Indies. Bentley found that he could produce a similar eruption on the skin of the arm by applying moist earth which had been contaminated with faeces containing *Ankylostoma* ova and incubated for a week, and that the same earth when incubated with faeces free from *Ankylostoma* eggs, or after the larvae had been killed by drying for a few hours at the air-temperature, produced no effect. He concluded that the eruption is primarily due to invasion of the skin by the larvae, though a secondary bacterial infection is doubtless usually also present. The fact that the disease occurs only in the wet season is easily explicable on this hypothesis, since the *Ankylostoma* larvae can only develop on wet ground. It appears that affections similar to or identical with the coolies' water-itch in Assam also occur elsewhere in tropical countries, though they may of course be due to other causes.

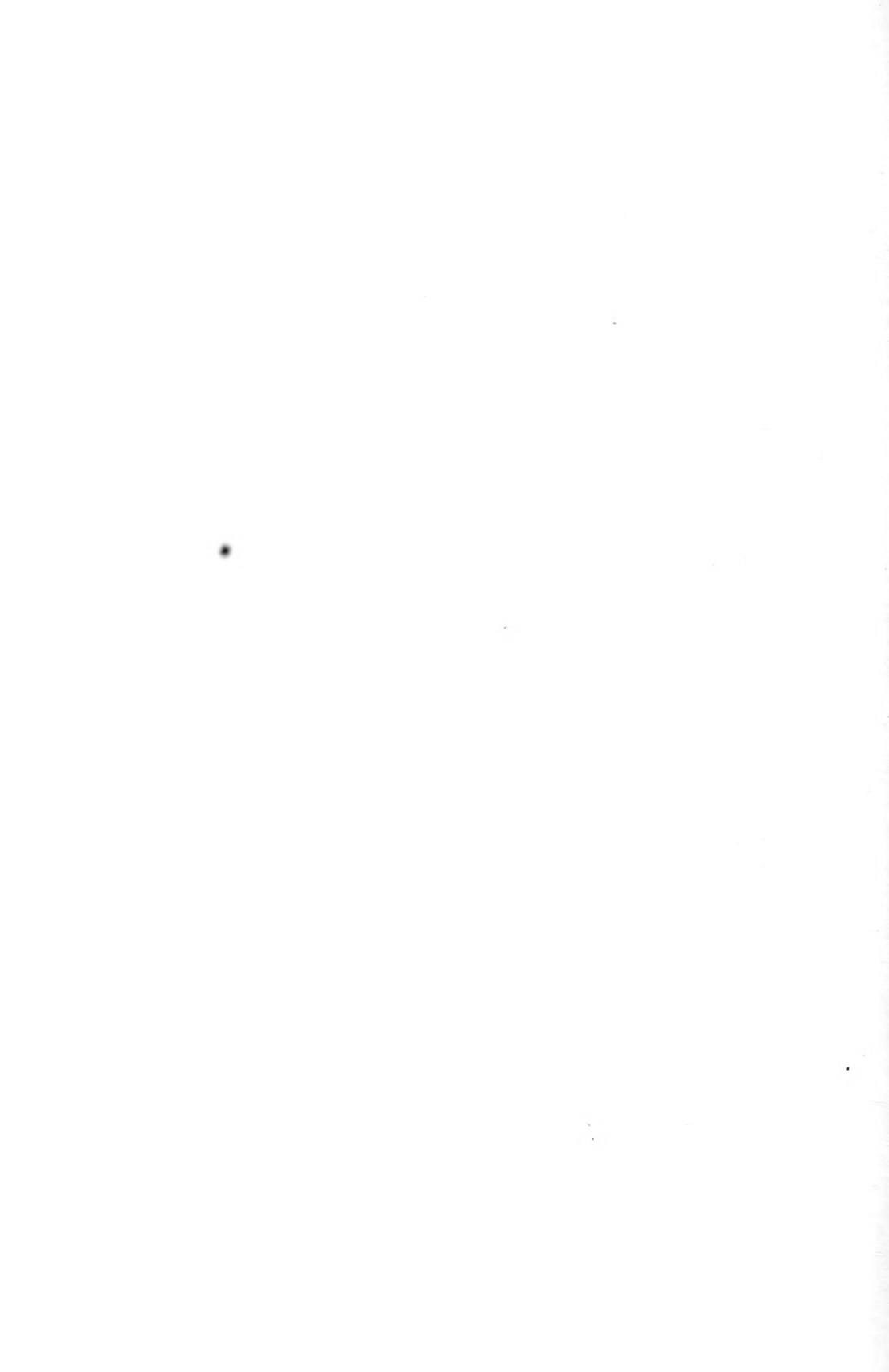
We have already given an account of the very troublesome skin-eruptions which have been associated with the prevalence of ankylostomiasis among the miners at Dolcoath mine in Cornwall, and which had come to be known as "New Sump bunches," the "New Sump" or Engine shaft at Dolcoath being the one in which the men were far most frequently affected by both anaemia and the skin eruptions. The eruptions did not specially affect the feet of the miners, and were usually on other parts of the body; but the miners wear thick boots, and often work stripped to the waist on account of the heat, or very lightly clad. They

¹ *Arch. Italiennes de Biologie*, Vol. 37, p. 269, 1902.

² *Brit. Med. Journ.* 1902, Vol. 1, p. 190.



A severe case of "bunches" on the arm of a Cornish miner. See pp. 88—89.



are thus more exposed to mud and dirt on other parts of the body than on the feet. Plate IX is from a photograph of a bad case of "bunches"; we are indebted for it to Dr Blackwood of Camborne. The patient was a Dolcoath miner who had come to him for treatment. The men described intense itching, followed by papular, vesicular, and pustular eruptions, just as in the case of "water-itch."

Skin symptoms have not, so far as we know, been described in connection with outbreaks of ankylostomiasis in mines elsewhere. Enquiries made recently by one of us among medical men engaged in dealing with ankylostomiasis in the Westphalian mines failed to elicit any information as to skin eruptions specially associated with ankylostomiasis. Some of the miners referred, it is true, to skin eruptions due to their work underground, but there was no evidence to connect these with ankylostomiasis. The infection of the Westphalian mines was, however, so far as could be judged, in no case nearly so intense as in the New Sump shaft at Dolcoath, and skin affections to the same extent could therefore hardly be expected.

In November 1902 one of us made numerous attempts to produce a skin infection on his own person by smearing cultures of larvae on the arm, where they were allowed to remain (bandaged up in a moist condition) for as long as twelve hours. Artificial cultures of all ages were used, including some which contained abundant active "encapsuled" larvae: faecal material containing larvae from Dolcoath Mine was also tried. In no case was any local reaction obtained; this is remarkable if the generally filthy nature of the applications is alone considered. From November 1902 till June 1903 both the faeces and blood were repeatedly examined and no evidence was found of the presence of eggs or of anaemia or eosinophilia. From June till October 1903 these examinations were repeated occasionally but always with negative results.

Quite recently Looss¹ has published a further most important paper on skin infection. Two puppies, which had previously by examination of the faeces been ascertained to be free from infection, were smeared over about 20 square centimetres of the back with a culture of the larvae of *A. caninum* (an intestinal worm closely allied to the *Ankylostoma* of man). The culture was kept applied for two hours by means of a bandage, and scrupulous precautions were taken to disinfect the place afterwards, and prevent the animals from by any means swallowing any of the larvae. Both animals died after nine days.

¹ *Centralbl. f. Bakt.* Vol. 33, p. 338, 1903.

The organs were very pale¹, and in the small intestine were found enormous numbers of the worms in an early stage of development. An experiment was also made on a man who was at the time free from infection. A small quantity of a culture of *Ankylostoma* larvae was smeared on his arm. The usual inflammatory reaction appeared at the time, and 71 days later ova began to appear in the faeces. These increased in numbers for several weeks, until they became fairly abundant. So far as could be ascertained the man had not been otherwise exposed to infection in any way.

These experiments seem to render it all but certain that infection with *Ankylostoma* may occur quite readily through the skin. Previously to the paper by Looss just referred to experiments had been published by Pieri² on infection through the skin. The latter observer obtained apparently negative results. He experimented with a culture of ripe larvae of *A. americanum* on three persons, including himself and Prof. Grassi, in whose laboratory he was working. After 40 days no ova could be found in the faeces of any of the persons experimented on, and the results appeared to be clearly negative, as it was assumed that, as in the case of infection through the mouth, the ova would appear within this period if at all. After 71 days, however, ova were found in the faeces of Pieri, who had been most strongly infected. Up to six months from the date of infection no ova were found in the faeces of the other subjects of the experiment. Pieri considers it probable that his infection was accidental, as he was working with cultures in the laboratory; but at any rate these experiments seem to partly confirm the theory of Looss, though they indicate that infection occurs much less readily through the skin than through the mouth in man.

Assuming the correctness of the theory of Looss it seems very probable that in the case of work such as mining or brick-making, and even agricultural employment, the chief path of infection is through the skin rather than through the mouth. If this be so it cannot be expected that precautions, however perfect, against infection through the mouth will avail in preventing infection with *Ankylostoma* during such work.

¹ In one of the animals there were some small haemorrhages in the mucous membrane, and in the other no haemorrhages. The young worms contained no blood. As, however, the signs of anaemia seem to have been very marked this experiment seems to confirm the conclusion which we put forward in our previous paper that the anaemia cannot be explained by loss of blood caused by the bites of the worms.

² *Arch. Italiennes de Biologie*, Vol. 37, p. 271, 1902.

There are evidently many points with regard to infection through the skin that remain to be cleared up. Why, for instance, was the period between the skin infection and the appearance of ova in the faeces so long (71 days) in the case of Looss's and Pieri's experiments on man, as compared with the much shorter period (about 30 days) in infection by the mouth, and the very short time required for the worms to reach the intestine in the experiments on dogs?

Van Durme¹ has recently found that the larvae of *Anguillula intestinalis* (*Strongyloides intestinalis*) are also capable of penetrating the skin of animals, and producing dermatitis with a pustular eruption.

Our knowledge is scanty as to the symptoms produced during the period which elapses between infection with the *Ankylostoma* and the complete development of the adult worm. The experiment of Looss, quoted above, shows that fatal effects may be produced in animals; but no definite symptoms have been observed in cases of artificial infection in man; and in natural infection in man the larvae are usually introduced so gradually that observations can hardly be made as to the early effects of infection. It is not yet even known whether the eosinophilia and anaemia begin to develop before the worms have reached their adult stage, though it is perfectly clear that the presence of the adult worms in the intestine maintains the anaemia, at any rate in some individuals. Men coming fresh to the work in the infected Cornish mines seem most apt to suffer from anaemia; and this might at first sight seem to suggest that the worm in its early stages causes most harm. Fresh infection must, however, be occurring, and the facts are most easily explained on the theory that in the majority of individuals some form of immunity is gradually established to the presence of the worms. Nearly all the men working in the mine appear to be infected with the worm, and a great many of these suffered at first from anaemia, but gradually recovered completely without giving up their employment and without treatment; on the other hand there were some individuals who continued to be ill for long periods even after giving up work underground, and in whom, therefore, the immunity did not become established, though they recovered when treated with thymol. The immunity theory seems also to be strengthened by the fact that in many tropical districts nearly all the inhabitants may be infected with the worm, while cases of actual anaemia are not common. Stiles mentions that the anaemia of ankylostomiasis seems to be less common

¹ Thompson-Yates Laboratory Reports, Liverpool, 1902, p. 471.

among negroes than among white persons. Possibly there is some degree of hereditary immunity in natives of tropical countries, though all races are certainly more or less subject to the disease.

Diagnosis of Ankylostomiasis and Ankylostoma Infection.

As only a certain proportion of the persons infected with the *Ankylostoma* show any symptoms of illness, a distinction must be drawn between persons actually suffering from ankylostomiasis and those who are simply infected with the worm, though from the standpoint of Preventive Medicine the diagnosis of *Ankylostoma* infection is usually much more important than that of the disease itself.

The symptoms which are suggestive of ankylostomiasis fall under two general heads:—(1) those belonging to the anaemia such as pallor, shortness of breath on exertion, and palpitations of the heart; (2) gastrointestinal symptoms, including dyspepsia of various kinds, epigastric pain, and irregularity of the bowels. This second group of symptoms includes nothing which might not be due to any anaemic condition, but there is little doubt that patients suffer from these troubles before (or without) becoming at all seriously anaemic. The skin eruptions which we have previously described (III, p. 107) may not be due to *Ankylostoma*, but there are, we think, sufficient reasons for looking upon them as, in part at least, specific, so that their presence should rouse suspicion. It may not be out of place if we here repeat the common warning—not to judge of anaemia by the colour of the patient's cheeks. Ruddy cheeks are often found with the pale lips and conjunctivae which are almost always trustworthy guides to the haemoglobin content of the blood. Definite anaemia in adult males without any obvious cause is such an uncommon affection that its occurrence should at once suggest Ankylostomiasis, especially in those whose occupation is suspicious (miners, &c.), and who have been exposed to infection in the tropics. We have found the haemoglobinometer of the utmost service in establishing the existence and degree of the anaemia, and in following the effects of treatment. An accurate determination of the haemoglobin can be made in three or four minutes with the Gowers-Haldane haemoglobinometer. This instrument requires, however, a supply of lighting gas for saturating the blood-solution with carbonic oxide; and as gas is not always available it is well to have, along with the instrument, one of the old Gowers picro-carmin standard

colouring tubes, the indications given by which may be checked from time to time against the CO-haemoglobin permanent standard of the Gowers-Haldane apparatus.

Assuming that ova have been found in the stools, and that the patient is more or less anaemic, there will nevertheless often arise doubts as to whether the symptoms presented by a patient are wholly, or even partly, due to ankylostomiasis; and this difficulty is much greater in tropical countries, where, perhaps, nearly every native of the country may be more or less infected. Such causes of secondary anaemia as malaria and other fevers, scurvy, chronic starvation, &c., must thus all be considered. In certain mines the possibility of lead-poisoning must also be taken into account. In Cornwall the chief source of difficulty in diagnosis was "miner's phthisis" or silicosis in its various forms. It was often impossible to judge whether respiratory and cardiac symptoms were due to ankylostomiasis alone, or partly to silicosis; but in cases of pure ankylostomiasis there was seldom if ever any wasting.

A positive diagnosis of *Ankylostoma* infection rests on the discovery of the eggs in the stools. In the majority of instances they occur in very large numbers, and owing to the fact that they are deposited when the contents of the gut are still fluid, they are uniformly distributed throughout the faecal mass. It is, in consequence, unnecessary to search more than one portion of a stool, and as a rule the eggs are very easily found. A small portion of the faeces should be thoroughly mixed with two or three drops of water in a watch-glass, and the fluid part removed to a slide and spread out into a thin layer by means of a cover-glass. Fluid stools may be examined direct, but in the end the quickest plan is to dilute the faeces and examine several preparations rather than to attempt to search in the thicker mixture. A thin, watery film can be examined very quickly and with a low power, and there is no chance of eggs being hidden by *débris*. In a thick preparation the search has to be made very slowly and carefully. The most convenient objective to use is one of about 8 mm. ($\frac{1}{2}$ or $\frac{3}{8}$ in.), though, using thin preparations and with some practice, the eggs can easily be found with a $\frac{2}{3}$ or 1 in. As in all similar manipulations, time is on the whole saved if every preparation is examined systematically on a mechanical stage.

It is fortunate that the eggs present an appearance which is highly characteristic, and there is really nothing else found in *fresh* faeces which can be easily mistaken for them. The important and diagnostic points of the egg as seen in fresh faeces are:—

- (1) the egg is regularly oval in outline and has a regular contour

(not circular as in *Taenia* or flattened on one side as in *Oxyuris* or with projections as in *Trichocephalus* or *Bilharzia*).

(2) the egg-shell is very thin and appears as a single line (not as two concentric lines as in *Oxyuris*, &c.).

(3) the egg-shell is smooth (not mamillated as in *Ascaris*). *Ascaris* eggs, stripped of their mamillated outer covering, have sometimes been mistaken for *Ankylostoma* eggs: the shell is, however, far thicker.

(4) the egg-shell is transparent and perfectly clear (it is not stained brown or yellow as in *Trichocephalus* or *Ascaris* with the outer covering not stripped off).

(5) the contents are grey (not yellow) in colour, are very readily seen through the pellucid shell, are divided into distinct roundish masses (*i.e.* cells), usually 4 to 16 in number, and do not completely fill the shell.

In any case of doubt the eggs should be carefully measured. Different authorities give varying figures¹; the average of all the measurements given by Scheube is about $59\mu \times 37\mu$, and unless the figures obtained are somewhere near these suspicion should be aroused that the objects in question are not *Ankylostoma* eggs. When the egg has been seen once or twice it seems almost impossible that any mistakes should be made: but W. R. Stone² has recorded that the seeds of the strawberry have been carefully collected from faeces and placed in a museum series as *Ankylostoma* ova.

A positive diagnosis is usually reached very quickly; but it is necessary to search several preparations before one is justified in saying that no eggs are present. When the eggs are very few Giles³ has suggested that the faeces should be mixed with a dilute solution of magenta, when the eggs stand out as unstained objects. We have not found this procedure of any particular advantage, but our experience of Stiles'⁴ sedimenting process is not unfavourable.

Even if no eggs can be found by prolonged and repeated searches, it does not necessarily follow that the patient does not harbour the worm (see above, Vol. III. p. 126). Other points must then be taken

¹ See the table in B. Scheube (*Diseases of Warm Countries*, Eng. Trans. 1903, p. 420). The extremes given here are: length 44 to 70, breadth 23 to 43. Our own measurements give 56 to 70 \times 37 to 50, with an average of $62 \times 42\mu$. According to C. W. Stiles (*Treasury Dept. U.S. Hygienic Lab. Bull.* 10, 1903) the eggs of the American species (*Uncinaria americana*) are a good deal longer (64 to $76\mu \times 36$ to 40μ).

² *Medical News*, Vol. 82, 1903, p. 682.

³ *Report on Kála-Azár*, Shillong 1890, p. 109.

⁴ *loc. cit.*

into consideration: exposure to infection, symptoms (if present), and the condition of the blood. We have already detailed the results of the blood examinations made at Dolcoath, and purpose to deal with the use of the stained film in the practical diagnosis of *Ankylostoma* infection in a future paper. In this place we would only say that (1) the absence of an increase of eosinophile leucocytes does not render the presence of worms impossible, and (2) the failure of an eosinophilia to disappear after specific treatment is not an indication that the worm is still present.

From the point of view of public health, the important point is to diagnose the presence of the worm irrespective of the presence of any of those gastro-intestinal and anaemic symptoms which constitute the disease ankylostomiasis. If any such symptoms are present they may suggest the examination of the stools, or, among an infected population, may make the diagnosis of *Ankylostoma* a probable inference, but one which will require definite proof before any drastic treatment is undertaken.

It has been shown by several observers that thymol will sometimes produce specimens of the adult worms in cases where no eggs have been found in the stool. Except in exceptional instances it is hardly justifiable to administer an adequate anthelmintic dose of thymol as a purely diagnostic procedure. It may, however, be necessary sometimes to treat a patient on presumption. To find the worms easily, it is necessary to bring about an early and copious evacuation of the bowels in order that the worms may not be digested after being killed. A large amount of faeces should then be mixed and thoroughly agitated with five or ten times its bulk of water in some deep vessel (a large coffee-tin answers very well) and the mixture allowed to settle for a couple of minutes. The liquid is then poured off, fresh water added and the process repeated. After the second washing the sediment (which is now practically free from any objectionable odour) is emptied out on to a dish or plate and carefully searched. The worms are opaque white, sometimes with a reddish tinge, the females being about half-an-inch long and the males a little shorter; they are positively identified by the two pairs of pointed "hooks" on the ventral surface of their mouth¹. The characteristic bursa at the tail end of the male is visible to the naked eye, and its details can be made out under a low power. With the exception of the other varieties of *Ankylostoma* there are no parasitic

¹ The American species differs in some particulars: *vide supra* p. 74.

worms which possess anything at all like the copulatory bursa of the male. Permanent preparations of the worms are best made by mounting them in Farrant's medium or glycerine without any fixation.

We have repeatedly observed that in cases where, to judge from the abundance of the ova in the faeces, a large number of worms must have been present, only a very few whole worms, and numerous fragments, could be found in the faeces when there had not been an evacuation of the bowels very soon after the administration of thymol. The worms are readily digested by the pancreatic ferment, and in these cases nearly all seem to have been digested, as the faeces afterwards remained free from ova.

It sometimes happens that one meets with lively young larval worms while searching for eggs. If the faeces are quite fresh these may generally be taken to be *Anguillula stercoralis* without any very serious chance of error. The same assumption may be made in older specimens if these have carefully been kept cool since deposition and have not been in such a position that extraneous worms could have crawled into them from without.

It will, however, be necessary sometimes to search for evidence of *Ankylostoma* in faeces which have been passed for a considerable and often indeterminate time. Such occasions arise when examining faecal deposits collected from a mine or some such place. The eggs can easily be found in deposits which from their general appearance, loss of odour, &c., may be judged to be at least several weeks old. They only differ from fresh eggs in the appearance of their contents, which are agglomerated into a granular mass on the death of the embryo: the shell retains its contour and appearance for a long time. Even if the temperature has been such that larvae have hatched out, a certain number of eggs can generally (we fancy always) be found, since some of the eggs die in the early stages. The empty egg-shells are very difficult to find. If, however, the sample contains small worms and no eggs, a number of possibilities arise. The small worms may be (1) *Ankylostoma* larvae, (2) larvae or adults of some other parasitic worm, (3) larvae of some free-living nematode which have entered the faecal deposit since it was deposited, (4) the adult form of some small nematode worm¹. The last are probably easily separated by the fact that they will show sexual

¹ We are assuming that the larvae found bear some superficial resemblance to those of *Ankylostoma*. Dipterous and coleopterous larvae can and do occur in these faecal deposits; they are easily separated by their segmentation, the presence of hairs, eyes, the elaborate mouth, &c. Unless the worm is unsegmented it cannot be a Nematode.

characters, the females containing ova, and the males having some penile apparatus, commonly (according to our own observations) two equal spicules. But their presence in a sample introduces a possible fallacy which it is necessary to guard against: the eggs of some of these free-living nematodes are not at all unlike *Ankylostoma* eggs, and when such are found in an old faecal deposit which has been exposed to possible contamination from the soil it is necessary to see that the eggs correspond in all respects with those of *Ankylostoma* before pronouncing that they belong to that worm¹. The adult worms and the eggs found may be *Anguillula*, but it is not possible to say with certainty that this is the case, unless one can find similar living larvae in fresh stools. It is generally possible to see readily if the eggs present have been laid by the worms which are found: if so, they cannot be *Ankylostoma* eggs. A case might easily occur where free-living nematodes had entered faeces which also contained *Ankylostoma* eggs: under such circumstances difficulty might be expected, but we believe that it is possible to make a certain diagnosis in most cases if due care is exercised. In the absence of both *Ankylostoma* eggs and of any adult worms, the satisfactory diagnosis of any larvae which may be found does not seem to be a practical possibility. The larvae of many nematode worms so closely resemble one another, and the published information with regard to the non-parasitic forms seems to be so scanty, that it is not worth while to enter into any material discussion of the subject. There are, however, three points which have come under our own notice and which may be helpful: (1) the larvae may easily be reared, and if they belong to non-parasitic worms from the earth sexual forms can be obtained, (2) the larvae of both *Ankylostoma* and *Anguillula* have regularly tapering tails, those of some other allied worms are rather abruptly truncated, and terminate in a filament, (3) *Ankylostoma* larvae are relatively more slender than many others, and (when developed) the external sheath is very much more conspicuous. The collection of a number of samples

¹ Some samples collected in a Shropshire mine by Mr John Cadman, H.M. Inspector of Mines (who kindly sent them to us for examination), were full of some Nematode belonging to the family *Anguillulidae*. The eggs were very similar to those of *Ankylostoma*, but were rather smaller (41 to 60×28 to 34 ; average $53 \times 32 \mu$), the sides were somewhat flattened by pressure within the mother's body, the contents filled the shell more completely; and the young larvae which were present in some of them were larger. Similar eggs were to be seen within the bodies of the adult females which were present. There is every reason for supposing that these were free-living Nematodes which had entered the faeces subsequent to their deposition in the mine. The larvae which were present showed that they were not *Anguillula*.

of faeces underground is very much easier than to obtain fresh specimens directly from the men, and, if *Ankylostoma* is prevalent, evidence of its presence can, we believe, generally be derived from such material. But it is quite clear that nothing found in these deposits can be taken as positive evidence except undoubted eggs of *Ankylostoma*.

In several cases we have found young active larvae in fresh stools (cases IV, VI, XIV, XXI). These resembled the larvae of the parasitic nematode *Anguillula* (*Strongyloides*) *stercoralis* (*intestinalis*)¹ and probably belonged to that worm, which is so frequently found in association with *Ankylostoma*. In none of the cases however were they at all abundant. It is very rarely that the eggs of *Ankylostoma* hatch within the alimentary canal, and on the whole it seems practically certain that they were *Anguillula*. It is to be regretted that a more careful examination of them was not made at the time. How far *Anguillula* is definitely pathogenic is not known: gastro-intestinal symptoms (especially a chronic diarrhoea) and anaemia have been attributed to it, but other writers regard it as harmless. Bücklers² has recorded an eosinophilia of 13·5 p.c. in a case of pure *Anguillula* infection; P. K. Brown³ 6 p.c., and Pappenheim⁴ 0·8 p.c. It is however clear that neither the symptoms nor the blood changes in our cases could have been due to *Anguillula*, since this worm was present in so few instances.

Anguillula eggs are similar to those of *Ankylostoma*, but practically never occur as such in the stools: they hatch within the alimentary canal and appear as larvae.

Treatment.

The procedure adopted in Cornwall was to give a purgative of calomel in the afternoon, and next morning (usually a Sunday morning if the man was at work) to give three successive doses of 30 grains each (2 grammes) of thymol as emulsion or in capsules at intervals of two hours. The patient was kept in bed and instructed to take no food except tea or coffee and no alcohol. After the last dose another purgative was given, castor-oil being, however, avoided. No unpleasant effects

¹ Good summaries of our knowledge of the life-history of this worm are given by W. S. Thayer (*Journ. Exp. Med.* vi. 1901, p. 75), and R. P. Strong (*Johns Hopkins Hosp. Reports*, x. 1902, p. 94). Strong's case had 0·1 to 0·3 p.c. eosinophiles but had some leucocytosis from an abscess at the time.

² *München. med. Wochenschr.* 1894, p. 22.

³ *Boston Med. and Surg. Journ.* cXLVIII. 1903, p. 583.

⁴ *Centralbl. für Bakteriöl.* xxvi. 1899, p. 608.

were observed. The patients were all treated at home, so that the results could not be so well observed as if they had been in hospital. All the men treated were anaemic and more or less ill, and in most cases the improvement following the treatment was very striking, although in one case which is quoted below, the improvement was only slow, in spite of the fact that ova had completely disappeared from the faeces.

This treatment is substantially what has been adopted by English medical officers in tropical countries all over the world. Thymol treatment seems to have first been successfully tried by Bozzolo during the St Gothard epidemic. The particular method followed is practically that recommended by Lutz, who has discussed the treatment very fully¹, and it was adopted in Cornwall on the advice of Sir Patrick Manson.

The following cases illustrate the effects of treatment :—

R. C., age 25. Miner at East Pool Mine. When seen in Nov. 1902 had been ill with pallor and dyspnoea for 12 months. During this period he had been vigorously treated with iron, arsenic and other drugs, including β naphthol and bone-marrow, but had grown steadily and progressively worse. He had been so ill that he had been at home for 7 months and for a fortnight had been unable to leave his bed. He was intensely pale and very weak: there was no oedema. Hb. 17 p.c. Eggs very abundant. He received thymol treatment on Nov. 21, Nov. 28 and on Dec. 19, as up to the latter dates his stools still contained eggs. On Dec. 10 Hb. = 25%, and on Dec. 16 he was well enough to go for a walk. On Dec. 18 Hb. was 36 p.c. On Feb. 2, 1903 Hb. = 60 p.c., and on March 10 he was returning to work with 84 p.c. Hb. He was now in excellent health, and shortly afterwards left England for the S. African mines.

J. S., age 51, was overman for shaft-work. Illness began in 1899 with general weakness, palpitation and dyspnoea. Gave up underground work, stopped at home for 6 months, and then, somewhat better, did light overman's work on the surface. He had marked general oedema in 1901. In Nov. 1902 he was still seriously ill and for some time past had made no further improvement. Hb. 48%. Eggs abundant. On Nov. 14, 264 worms were obtained after thymol; patient said he felt better after this but on Dec. 1 his Hb. was only 43% and plenty of eggs were still present. During the winter patient made no great improvement though he felt distinctly better and his gastric symptoms were relieved. In the early part of 1903 he received two further dosings with thymol and during the spring and summer made good but slow progress. In the summer his faeces were twice examined by one of us with negative results, and in Dec. 1903 a very thorough search failed to reveal any eggs. His Hb. in July was 64%, and on Dec. 3, 92%.

P. P., age 43. Worked formerly as an ordinary miner at Dolcoath mine. Illness began 4 years ago with palpitation and dyspnoea, also had "bunches" badly. At home 5 months, when he improved considerably, but has only been able for light employment on the surface. Is pale, weak, more or less emaciated, and very

¹ Volkmann's *Sammlung klinischer Vorträge*, No. 265, 1885, p. 2478.

depressed. Has some of the appearances of commencing miner's phthisis. Hb. 35% on Oct. 31, ova fairly abundant in faeces. On Dec. 8 came to the Radcliffe Infirmary, Oxford, where he was under the care of Dr Ritchie. Very careful examination of lungs, heart, and other organs disclosed no abnormality. On Dec. 16, and again on Dec. 26, Hb. = 44%. On Dec. 26 three 30-grain doses of thymol after a purgative. No faeces till Dec. 28, when only about 11 half-digested worms could be found. Jan. 5, Hb. = 51%. Very few ova present in the faeces. Jan. 10, thymol treatment repeated. About 17 worms or parts of them found in the faeces. Jan. 13, Hb. = 62%. Jan. 19, repeated search shows no more ova in the faeces. Feb. 7, Hb. = 71%. Feb. 10, Hb. = 73%. Patient went home very greatly improved, and was now quite bright and cheerful. He had, however, only gained 5 lbs. in weight in spite of careful feeding, and he still looked very thin. Dyspnoea and palpitations much less, but still distinctly present. He found himself unable for hard underground work; and although he continued well for the next few months we hear that he has recently developed a bad cough and other symptoms of lung trouble.

The medicine which is universally used at the Westphalian collieries for treating ankylostomiasis is ethereal extract of male fern. As the doctors engaged in treating the disease are nearly all under the direction of Dr Tenholt, the Chief Medical Officer of the Knappschafts-Verein, the procedure employed is fairly uniform. Dr Tenholt informed one of us that as a general rule the following plan is adopted:—

On the first day, the patient, who is kept within the hospital, receives in the evening a purgative, consisting of 0·3 gramme (5 grains) of calomel. Next morning he takes no food, and is given 8 to 10 or 12 grammes (2 to 3 drachms) of extract of male fern, the taste being usually concealed by the addition of syrup of senna and a little chloroform. In the afternoon a further dose of 0·3 gramme of calomel is given. The patient is only kept in bed if he is weak. On the third day no medicine is given, except a further dose of calomel in the evening if required. On the fourth day the dose of male fern is repeated in the morning and the calomel in the afternoon. On the 5th, 6th, and 7th days the faeces are examined for ova, and if on three successive days no ova are found the patient is discharged. If ova are found the whole course is repeated from the beginning, and at the end of another five days the examination is repeated. If the result is again positive there is another week of treatment and examination. If at the end of this time ova are still present the patient is discharged for the time, as further treatment would be unwise. He cannot, however, return to work underground.

Such energetic treatment is, of course, not without its risks; but although several thousand men have been treated no deaths are known to have been caused by the treatment. There have, however, been two cases of permanent blindness, several cases of temporary affection of

vision, and a few cases of alarming collapse. Dr Tenholt remarks that some of the cases in which very few ova were present in the faeces have been most resistant to the medicine.

The opinion of both Dr Tenholt and Prof. Loebker is that extract of male fern is preferable to thymol, provided that care is taken that the extract employed is fairly fresh. Experience with thymol did not seem, however, to have been very extended in Westphalia.

It is clear enough that both extract of male fern and thymol are very effective remedies, and there is little doubt that one or the other will continue to be preferred by different doctors. Thymol, has, however, the advantage that it can always be obtained in good condition. It has been used very extensively by English medical officers in Egypt, India, the West Indies, West Africa, &c., and seems to have given very satisfactory results.

To obtain the desired result it is necessary that the drug should be well powdered and administered in sufficiently large doses. Doses of two or three grains are quite useless. The doses necessary are so large that their administration would be attended with no inconsiderable risk if any material proportion were absorbed from the bowel. An amount sufficient to turn the urine dark brown or black is often absorbed when 90 grains in all are given. To prevent this reaching a dangerous quantity one must be careful to avoid giving anything to the patient in which thymol is soluble. It is for this reason that alcohol, oils, and fats should be withheld; above all the drug itself must not be given dissolved in alcohol. Sandwith has found that brandy does no harm, and is of use with very reduced patients, but it is better not to run any risk of occasionally meeting with someone who shows an idiosyncrasy towards thymol. The experiments of Stiles and Pfender¹ show that the administration of alcohol with thymol reduces the minimum fatal dose for dogs.

Whatever anthelmintic is used, it is of great importance that the results of treatment should be properly gauged by microscopical examination of the faeces. Thymol is sometimes without effect, and, though a partial clearance of the worms will nearly always benefit the patient, a complete cure cannot be looked for unless all the worms have been killed. As an infected individual, a partially cured patient is more dangerous than one who is manifestly anaemic and incapacitated from work. To secure this complete destruction of the worms it is frequently necessary to repeat the thymol or other treatment more than

¹ Stiles, *loc. cit.* p. 87.

once. In Cornwall the very few cases which are known to have been treated to a cure (*i.e.* till no more ova could be found in the stools) have all required more than one dose. Of the 184 cases recorded by Sandwith¹ only 23% were cured by one dosage with thymol, and 23% required more than three dosages. The largest number necessary was eight, and the average number 2.6.

In some instances thymol fails to kill the worms at all. C. F. Fearnside² gives some examples where daily doses of 20 or 30 grains continued over long periods left many eggs still in the stool; we have had one case where after 40 grains no worms could be found in the faeces: subsequently 60 grains produced large numbers.

Thymol, again, may kill all the worms, but may yet fail to save the patient's life. Cases where the severity of the disease has so destroyed the patient's constitution that the process of reparation fails despite removal of the cause will be chiefly found among those who are subjected to the concurrent ravages of some other chronic disease, or chronic starvation.

Sandwith had eight cases which died after thymol treatment, and T. A. Claytor³ has recorded a case which proved fatal, although the condition of the blood had much improved under an extremely vigorous and apparently successful course of thymol.

In estimating the efficacy of treatment, it is necessary to wait a few days before examining the stools. Some worms may still be present, but may not be laying eggs freely immediately after having been subjected to the action of an anthelmintic. That this may actually occur has been shown by Bücklers⁴.

We cannot too strongly emphasize the fact that it is most undesirable to undertake the treatment of a case unless one is prepared to give adequate doses, and to repeat such doses at intervals until the faeces are found (on thorough examination) to be free from ova.

Prevention of Ankylostomiasis.

It is evident that the problem of preventing the spread of ankylostomiasis differs greatly in warm climates where the disease is endemic among the general population, and in temperate climates where it is

¹ *Lancet*, 1894, i. p. 1365.

² *Brit. Med. Journ.* 1900, ii. p. 541.

³ *American Journ. Med. Sciences*, cxxiii. 1902, p. 28.

⁴ *loc. cit.*

only met with under special circumstances, as in underground work. In the latter case the task of prevention is much less difficult.

It follows from the account given above of the life-history of the *Ankylostoma* that to prevent the spread of ankylostomiasis it is necessary to prevent the ripe larvae from entering the body. This end might be attained either by personal precautions against ingestion of larvae by the mouth and skin infection, or by preventing the development of larvae. We may first discuss what may be obtained by the former means.

There is no evidence, and very little probability, that larvae may be present in water which to naked eye examination is perfectly transparent. If larvae are brought into water they rapidly sink to the bottom, and are therefore very unlikely to be present in clean water, since the finer sediment with which they are abundantly mixed in the polluted mud or dirt where they develop settles more slowly than the larvae themselves. We believe that there is practically no risk of infection if care be taken that only clear water is drunk. Among the native populations in some warm countries even this elementary precaution is not, however, taken, muddy water being sometimes drunk, so that as a matter of fact ankylostomiasis is probably spread to a considerable extent by water. So far as we have been able to ascertain the underground water in mines is very seldom drunk by miners in European countries, the almost universal custom being for miners to take sufficient water, tea, or coffee with them from the surface, unless water from the surface is supplied by special pipes, or there are springs unconnected with workings above. The water which drains from a mine is evidently liable to pollution of all kinds, both organic and inorganic, and ought never to be drunk.

Ankylostoma larvae may also be ingested with dirty food, and absence of ordinary precautions in this respect is doubtless often responsible for infection. In mining work it is often difficult for the miners to properly clean their hands before taking their underground meals. The food can, however, be kept wrapped up in paper when handled, and by this means kept from contamination. This simple and effective precaution, which is very commonly adopted by miners, ought to be always taken when food has to be eaten with unwashed hands.

Infection is probably often carried by introducing dirty hands into the mouth, by dirty pipes, &c., and this would be prevented by more cleanly habits.

Air-borne infection seems to be very improbable. In the first place

living *Ankylostoma* larvae are naturally found only in damp material, which cannot be suspended as dust. If this material is dried and thus becomes capable of suspension in air, the larvae are killed by the drying. In any case they are so bulky that if by any means they were temporarily suspended in the air they would rapidly fall to the ground.

In mining and other similar work it appears to be probable that the most serious risk of infection is through the skin, and the nature of the work makes it quite impossible to avoid this. European miners wear thick boots, so that their feet are but little exposed; but the high temperatures often make it impossible for them to work unless they are lightly clad, and often they have to work stripped to the waist. Temperatures of from 80° to 95° are, for instance, common in Cornish mines. In any case their thin clothing is probably very little protection against *Ankylostoma* larvae, as their clothes are very often wet with mine water or perspiration, so that the larvae will easily penetrate to the skin. Practically the only precaution that can be taken is to avoid unnecessary contact with mud, dirty water, or damp surfaces which are likely to be contaminated with larvae; and doubtless much may be done in this way. The contaminated soil is, however, liable to be carried all over a mine, including the ladders used in metalliferous mines and in coal-mines where, as is common in Westphalia, the strata run almost vertically up and down. The larvae may find their way on to damp wood, and the Dolcoath miners often attribute their "bunches" to contact with damp wood.

This brings us to the consideration of measures designed to prevent the development of larvae in places where they might give rise to infection. One method of attaining this object is to establish a rigid system of excluding persons infected with the worm from the places where the development of larvae would entail risk. To exclude such persons it is necessary to institute a medical examination of everyone admitted, and exclude all those whose faeces contain ova. To exclude only those who are evidently anaemic would clearly be of little use from the point of view of prevention, since for one person who is actually anaemic there will usually be a large number who are infected though not evidently suffering in health. In the most infected Cornish mine, for instance, it appeared from our observations that practically speaking all the men were infected, while comparatively few were markedly anaemic. Many of the infected men had a completely normal percentage of haemoglobin. The same observation was made on a much larger scale in the Westphalian colliery district, where complete

statistical information was obtained with regard to many of the mines¹. It was found that while in nine of the most seriously affected collieries only 958 men had been noticed at the periodical medical inspections during 1902 to be visibly anaemic, yet ova were present in the faeces of 6,190 men at the end of the year. In consequence of the general alarm produced by the spread of infection among the miners it was enacted in 1903 that in addition to sanitary precautions underground the faeces of 20 % of the miners working in each colliery should be examined, and that when as a result of this examination a mine was found to be, in the opinion of the Chief Inspector, infected, the faeces of all those employed in it should be periodically examined, and the infected men excluded from work until they had been completely freed by treatment from the worms, and satisfactory evidence from a specially qualified physician was forthcoming that no ova were present in their faeces. Special doctors were attached to each infected mine, with the sole duty of conducting the microscopic examinations and treatment. Barrack hospitals were also provided for the treatment of those infected, and the certificate of freedom from infection was only given after examination of the faeces on three different days, as it was found that a negative examination on one day did not afford sufficient evidence.

The effects of these regulations, which were very thoroughly carried out, have been most marked, as might be expected. In every colliery where they were applied the percentage of infected men has greatly diminished. Thus in six large collieries for which complete figures were recently available the average percentage of infected men had diminished from 30 % at the first round of examination and treatment to 8·7 % at the third; and cases of anaemia had apparently disappeared entirely. At another colliery the percentage of infected men was 20 % at the first examination, 13 % at the second, 8 % at the third, 3·6 % at the fourth, and about the same at the fifth, sixth, and seventh examinations. The latter observations seem to indicate that it is difficult to completely eradicate the infection. Official statistics which have just been published show that out of 188,730 men employed in the mines of the district 17,101, or 9·1 %, were infected. In the collieries declared to be infected

¹ For recent detailed information with regard to the Westphalian outbreak see the Report by Dr Haldane to the Home Secretary on Ankylostomiasis in Westphalian Mines, *Parliamentary Paper* [Cd. 1843], 1903: also special supplement to the *Colliery Guardian*, November, 1903.

out of 63,000 men 12,157 were found to be infected, or 19·3%. By December 1903 this number was reduced to 4,819, or 7·6%, although in most of these collieries only one round of examination and treatment had been completed. Considering that at the time when the measures just referred to were initiated the disease was rapidly spreading, this is a most striking result.

The inconvenience and expense of carrying out such regulations is of course very considerable. In Germany however the matter is greatly facilitated by the fact that there is established by law a very complete medical and insurance organisation for the miners, with ample funds, which are obtained by deducting a certain amount weekly from the miner's wages and the mine-owner's profits. The yearly income of this association in Westphalia is about £1,500,000, the number of members being about 200,000. This organisation has provided doctors and hospitals, and its certificates must be produced by men applying for work, so that infected men can easily be traced. A great deal of the expense falls however directly on the colliery-owners, who have paid part, at least, of their wages to the men confined to hospital for treatment, besides other heavy expenses. The treatment and subsequent examinations occupy at least a week, but often much longer, as repeated doses of vermifuge remedies are commonly needed in order to completely expel the worms. The men not unnaturally object to the treatment and confinement to hospital, particularly as they themselves seldom feel in the least out of health, and the treatment is far from pleasant and occasionally causes symptoms of collapse and impairment of vision. Several cases of permanent blindness or impaired vision have been observed in Westphalia as a result of large doses of male fern.

The difficulty of carrying out this system would certainly be greater in England and elsewhere than in the Westphalian mines; and the question arises whether it is not possible to obtain almost equally good results for practical purposes by easier and less expensive means. The Westphalian system of inspection and treatment has been combined with very thorough sanitary precautions underground, which seem to be necessary in any case, and which must now be discussed.

It is clear that if contamination of the ground by human faeces can be altogether prevented there is no possibility of ankylostomiasis spreading, as ripe larvae cannot then reach those who would otherwise be exposed to infection. Ankylostomiasis seems to be entirely absent as an endemic disease in towns with any moderately good system of

removal of excreta. This is the case even in countries such as Egypt and the tropical parts of the South American Continent, where the disease is very prevalent in the agricultural districts. There may be great difficulties in carrying out efficient measures for the safe disposal of human faeces among the native populations in tropical countries; but in European and North American countries at any rate such measures ought not to present much difficulty. The state of matters disclosed by Stiles in the case of country districts in the Southern States of America might, for instance, well be remedied by suitable sanitary administrative measures. The same remark applies to the case of mines and brick-fields in North European countries.

So far as we have been able to ascertain it is a very general custom among miners in England to deposit their faeces at any convenient place in the mine. Some miners, it is true, very seldom make use of the mine for this purpose, but others do so regularly. We are informed that in some collieries the men usually bury the faecal deposits in coal-dust in the "goaf" (worked-out part of the mine); but it is quite common to find these deposits at the sides of haulage roads in wet places, and elsewhere in positions where they might easily be trodden on and thus carried about the mine. In England there are no legal regulations of any kind with regard to pollution of mines by faeces.

Some mines must be much more liable to become infected than others. It follows from what has been said above that when a mine is naturally, or on account of very free ventilation, dry, the larvae can hardly develop to the infective stage. In all mines, however, wet parts can be found; and pumping is always needed to keep the workings from being flooded.

In some mines, again, the temperature may be so low that development of the larvae only occurs very slowly, if at all. There are, however, very few mines of any size in England where a temperature of over 16° C. (61° F.) is not met with in many parts of the workings; and it has been shown above that the infective larvae can develop at this temperature. Experience hitherto in Westphalia seems to indicate that there is much less likelihood of a mine becoming seriously infected if a temperature of over 21° (70° F.) is not reached at the working face; but both laboratory experiments and the data obtained in the course of the Belgian Government Enquiry on ankylostomiasis show that mines with a much lower temperature may become infected. Out of 41 infected mines in the Liège District the temperature at the working face is

stated as under 20° (from 15° to 20°) in 16 cases¹. The percentage of infected men varied from 4·4 to 5·2.

In Levant Mine near St Just in Cornwall the temperature is very high, ranging from 23° (71° F.) at the bottom of the down-cast shaft to 34·5° (93°) in the deep workings. The workings are also very wet. Yet there are no symptoms of ankylostomiasis among the miners, though it seems almost certain that infection has been repeatedly introduced. The workings are under the sea, and the mine-water is salt. A sample was found to contain 1·8% of sodium chloride. In this water we found that newly hatched larvae (though not the ripe "encapsulated" larvae) were killed; and probably the freedom of the miners from ankylostomiasis is due to the salt water. In some other mines similar causes may prevent the growth of the larvae.

In spite of the fact that some mines may doubtless be practically immune from infection with *Ankylostoma* larvae we think there is urgent necessity for measures to prevent pollution of the workings of any mine by human faeces. Even if there be no risk of ankylostomiasis through the absence of such regulations, there is certainly risk of the spread of other infective diseases such as enteric fever. For this reason, and because in practice it is evidently very difficult to say whether or not any particular mine would be immune to ankylostomiasis, we think that the regulations should be made legally compulsory. A further reason in favour of making no exceptions is that when only certain mines come under regulations of this kind difficulties are more apt to arise with the miners, who are accustomed to move freely from one mine to another.

To carry out the object of preventing pollution of a mine by human faeces it is necessary to prohibit such pollution under a sufficiently deterrent penalty; we have no doubt that the great majority of miners would support the regulations, but there are always some, particularly among the younger and thoughtless men, who could only be restrained by the fear of punishment in some form.

As a rule there is no reason why any miner should require to relieve his bowels underground except very occasionally, and the habit which some miners have of doing so should be put a stop to. The necessity will, however, sometimes arise; and arrangements must be made to provide for this. In the Westphalian mines galvanised iron receptacles,

¹ Barbier. Paper read before the International Congress of Hygiene, Brussels, 1903, Section IV. The figures are given in the Table at the end of the paper.

furnished with a seat and air-tight cover, are placed in convenient positions along the roads and in the workings of each mine, the duty of providing and keeping them in order being thrown on the mine-owners. At Dolcoath Mine in Cornwall galvanised iron pails of a convenient height are now provided, but without seats, since the latter are apt to become soiled. In Westphalia milk of lime, which kills the larvae with certainty, is generally used as a disinfectant. It has, however, the disadvantage that it does not act as a deodorant; and the smell from the receptacles is very unpleasant. At Dolcoath dry disinfectants have hitherto been used for the pails; but information as to the best practical disinfectants has not yet been obtained. Many substances are probably available. The experiments quoted above show, for instance, that 5% izal emulsion will kill even the "encapsuled" larvae. Strong-smelling disinfectants are scarcely available in a mine.

Any legal restriction as regards pollution of the ground by miners would require to be accompanied by provisions requiring mine-owners to provide and keep in order a sufficient number of underground receptacles for the reasonable requirements of the miners. It is also desirable that a sufficient number of good privies or water-closets should be provided on the surface, though the need for this is not so great at English collieries as in many Continental mines, where the men change their clothes and wash in a building provided at the pit-head. We can see no reasons why the regulations just recommended should not be successfully carried out in all mines; and where they were carried out the infection of a mine with ankylostomiasis and other similar filth diseases would be practically impossible.

In the case of mines which have already become thoroughly infected there is much to be said in favour of the Westphalian plan of continuing for some time to examine and treat every man in the mine for infection with the *Ankylostoma*, and at the same time introducing strict sanitary regulations and endeavouring by every means to destroy the larvae already present. The practical difficulties of carrying out such a plan in any mine in Great Britain are, however, very considerable, and would require to be carefully taken into account beforehand. One of the first results of introducing such a plan might probably be to disperse the miners to other mines and to prevent other men from applying for work. On the other hand, when a mine is once thoroughly infected, the men employed in it will continue for many years to be a source of danger to other mines; and the infection will be got rid of very slowly by sanitary

measures, however perfect, since the adult worms, and possibly even the larvae, may continue to live for some years.

Men who are suffering from symptoms of ankylostomiasis ought never to be allowed to work underground unless they submit to efficient medical treatment. They are not only an evident source of danger to other miners, but they may easily become so ill as to be incapable of providing for themselves and their families, even if they are not in actual danger.

In the case of mines which are not infected, mine-owners would do well to exclude as far as possible infected men. Any new men coming from infected countries or districts might well be examined medically before being allowed to work underground. By this means the risks of infection would be considerably diminished, though by no means abolished. Local arrangements might easily be made for the examination of samples of faeces from suspected men.

In some cases it may be possible by increased ventilation to so dry a mine or reduce the temperature that the development of ripe larvae is impossible; and doubtless the excellent ventilation in most English collieries greatly reduces the risk of ankylostomiasis. In such cases, however, not merely expense but also other factors have to be taken into account. For instance, in metalliferous mines the dangers from inhalation of stone-dust completely dwarf those from ankylostomiasis; and a dry mine, which would probably be more dusty, might be far more dangerous to health than a wet one. In coal-mines the risk of coal-dust explosions has also to be considered; and to guard against these the Westphalian Board of Mine-inspectors, supported by the Mine-managers, have deliberately continued the system of keeping every working part of the mines damp, although they are well aware that by doing so they greatly increase the chances of the mines becoming infected with ankylostomiasis.

Addendum. We have just received an important paper (*Klinisches Jahrbuch*, Vol. XII. 1904) by Dr H. Bruns, Director of the Bacteriological Laboratory, Gelsenkirchen, on "The Influence of Physical and Chemical Agents on the Ova and Larvae of *Ankylostoma*." This paper not only contains a description of experiments referred to above, the results of which were kindly communicated to one of us by Dr Bruns, but many additional data of much interest. Bruns found, for instance, that after 5 days at 5° to 7° C. all ova and larvae were destroyed; that larvae were killed by 3 days, and ova by more than 16 days, of deprivation of

oxygen ; that larvae died after 20 days in clean tap-water ; that dilution of faeces up to from 10 to 100 times favoured the development of ova, while dilution to 1000 times or more prevented their development ; that while average faeces contain about 80 % of water, in faeces dried till only about 60—70 % of water was left ova would not develop ; and that ova were killed when only 40—45 % of water was left. He also describes a large number of experiments with various disinfectants, and mentions that many attempts at practical disinfection underground by spraying disinfectant solutions had been unsuccessful. These trials were made by himself and Mr G. A. Meyer, the well-known Manager of Shamrock Colliery in Westphalia.

NOTES ON BERIBERI IN THE MALAY PENINSULA AND ON CHRISTMAS ISLAND (INDIAN OCEAN).

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(*Beriberi Commission of the London School of Tropical Medicine.*)

(With 1 Plate.)

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I. Introductory.

IN the following pages a number of notes of personal observations are given; with these are some records and observations which have been communicated to me by the kindness of the observers. The literature of beriberi is so great, and at the same time so divergent, and so contradictory are the views and records that have been given, that I have excluded it as far as possible from these notes. When the day of complete knowledge of the nature of beriberi shall arrive it must assuredly happen that many of the hitherto published records will disappear from their failure to mention the circumstances which attended the real cause. Until this time is reached, copious reference to these papers becomes mere picking and choosing what seems to agree plausibly with the writer's ideas, to the exclusion of other possibly most important material.

II. Conditions of Life affecting the Prevalence of Beriberi.

(1) *Insufficient, sufficient, and supersufficient Diet.*

Much has been written on diet in association with beriberi, and the nitrogen starvation theory is well known. Writers on these matters do not appear to have distinguished between the sufficient and the supersufficient in diet, and have confined their attention more to the insufficient and the sufficient. In and about the Federated Malay States one could not help being struck by the circumstance that the well-to-do escaped the disease whilst those that were not well favoured were stricken; and it was difficult not to believe that some constituent in food, when in sufficient amount, had a preventive action, or perhaps,

to put it more accurately, that the presence of an adequate amount of some constituent of the diet had a sheltering effect on the nervous system of the individual. On the one hand, beriberi may spread through a community which is taking a sufficiently nitrogenised diet, according to the physiological standard, as for instance among the long-sentenced prisoners in the gaols of the Federated Malay States. On the other hand, in an endemic focus of beriberi, the disease may be temporarily at a lull, although the diet for some months has been below the physiological nitrogenous standard, as was the case among the coolies on Christmas Island for a certain period¹. These two contrasting circumstances must, however, be considered fortuitous; and they tend to show that beriberi is comparatively independent of relation to a diet which circles near about the physiological limits. When, however, persons are upon a supersufficient, in place of an insufficient or merely sufficient, diet there seems to be a marked resistance to the beriberic agency. Thus the well-to-do Chinese in the Malay States practically escape the disease entirely; in the tin-mining districts, where the deposits are rich, where the coolies earn much money, where the communications are good, and where the supplies of food are abundant, beriberi is a rare or absent disease.

i. *Christmas Island.*

A good instance is to be seen in the distribution of the disease amongst the Chinese on Christmas Island; they may be classified as follows:

(1) Coolies; (a) Sinkhehs or one-year contract men; these men have an advance money debt to work off, and consist of new men from China. They receive \$12.00 per annum wages.

(b) Lowkhehs, men who have stopped on for a further period at increase pay (\$7-8 per mensem), and no advance to work off.

(2) "Tukangs" or artificers (carpenters, blacksmiths, etc.), a superior class to the cooly and well paid. [They sometimes spent as much as \$2.00 on a single fruit-pigeon.]

(3) Cooks.

(4) Mandors or gangers.

(5) Contractor's agent and head men.

(6) Hylam "boys" or servants of Europeans.

¹ I should add that the Company's officials have taken steps to prevent a recurrence of this, which was due to the dilatoriness of the Chinese contractor.

All of these with the exception of the last two classes (Class 3 have a house to themselves) live in similar houses (Plate X., Fig. 3), on a fine, dry, open site overlooking the ocean (Plate X., Fig. 1), so that there is no palpable difference in surrounding for the groups. Class (5) live on the same terrace but in slightly differently constructed houses, whilst Class (6) live in the back premises of the Europeans' houses in less healthy positions.

It is a striking fact that beriberi was practically limited to the coolies, who alone were subsistent upon the contract diet. The only other persons affected amongst the Chinese workers of the Company were a few of the tukangs. The following figures illustrate the relative escape of the latter; in the year 1901, whereas there were more than 260 *deaths* from beriberi amongst the coolies, only 6 *cases*, with one death, were recorded amongst the tukangs; in the following year up to October there were 82 deaths amongst the coolies, and only one, a fatal case, is recorded in the whole year amongst the tukangs. It should be noted that there were roughly about ten times as many coolies as tukangs (say 500 to 50).

The contrast is also illustrated by a collective investigation which I made in December 1901; out of 443 coolies (with a small number of mandors) not in hospital, 146 had diminished or absent knee-jerks, and 52 markedly increased knee-jerks, a total percentage of about 45; moreover, just on 10% showed oedema of the shins which was apparently independent of other causes than beriberi. On the other hand, out of the 55 tukangs, 4 only had increased, and 4 diminished or absent jerks, and one only had a suggestion of beriberic oedema of the shins. The tukangs obtain their own victuals, whilst the coolies live on the contractor's supply, which in the case of the lowkhehs may be added to by their private means (especially pork and pigeons).

ii. *Pudu Gaol.*

The following figures, from the records of the Selangor gaol at Pudu, suggest something more than a mere coincidence of low deathrate and of more liberal dietary in beriberi; they are the more worth quoting since some figures have already been published which give a very misleading idea of the prevalence of the disease during the year 1902. I am much indebted to Dr Travers and Mr Galloway for preparing these figures which give the monthly admissions to hospital and the deaths; the former appear too large in so far as admissions and readmissions are

not distinguished, but at any rate during the year 1902 they are too small, for there were numbers of undoubted cases of beriberi which were not admitted, indeed a very high proportion of the prisoners was at one time affected. Attention must be called to the period of eight months from May to December 1901, during which more or less at the suggestion of Dr Hamilton Wright the prisoners were given some 6 ounces of meat every day irrespective of grade; from enquiries that I instituted it appeared that no fish (dry or fresh) was used. It will be observed that during the period there was no alarming increase of admissions, moreover there were only two deaths, a figure which with one exception is the lowest on record in the history of the prison. On January 1st, 1902, the ordinary grade diets were resumed; unfortunately we have no reliable information as to whether any cases of beriberi really arose in the gaol during these eight months. Soon after the return to the old order of things there was an extraordinary rise in the number of admissions in the month of January. Mr Galloway kindly analysed these for me with the following result:—

	December 1901	January 1902
	6 admissions	9 admissions
1st week		
2nd „	4 „	12 „
3rd „	4 „	16 „
4th „	4 „	31 „
Total	18	68

There was then an alarming increase in beriberi which really began to assume serious proportions some three weeks after the resumption of the ordinary diet arrangements. As will be seen from the table the disease became more and more prevalent, but the figures would be still more striking if all the cases which were merely put on light labour were included. By the end of the year the deaths had exceeded all previous records. Towards the end of the year the disease began to disappear, so that at the end of the following February there were no cases in hospital. Dr Travers wrote to me privately on June 19th, 1903, that there had not been any further cases in the gaol, though there was no corresponding diminution of the disease outside.

At first sight it might appear that the reintroduction of the dried fish had something to do directly with the recrudescence of the disease. But this, as in the case of the Richmond Asylum, Dublin, does not appear to be the case, since a number of those who were stricken did not partake of it. Quite a number of short-sentenced prisoners were

	1895	1896	1897	1898	1899	1900	1901	1902	1903
	Admis- sions	Deaths	Admis- sions	Deaths	Admis- sions	Deaths	Admis- sions	Deaths	Admis- sions
January	1	—	31	8	22	2	12	—	68
February	1	—	15	5	10	1	15	—	42
March	1	—	24	3	9	—	15	1	62
April	3	1	3	1	10	—	7	1	80
May	7	2	36	5	1	—	5	—	93
June	—	1	39	3	1	—	3	—	83
July	1	—	22	1	—	—	11	—	67
August	6	1	35	2	1	—	18	—	76
September	51	14	35	6	2	—	20	2	131
October	38	7	35	8	11	4	17	—	99
November	21	6	51	16	3	—	21	1	35
December	28	2	7	—	4	—	38	2	55
Total	158	34	275	54	73	2	180	7	891
Deathrate	21.5	8.75	19.5	2.75	9.5	3.9	2.5	6.25	

Feb. 24, 1903. No beriberi patients in hospital.

June 19. "There has not been a case of beriberi for nearly 4 months." Dr Travers.

Note I. From May to December 1901 all prisoners on special diet.

Note II. The figures for admissions in 1902, though including readmissions as in other years, do not represent the total number of beriberi prisoners, inasmuch as there were very many cases which were excused hard labour, etc. but who were not ill enough to be admitted to hospital.

Note III. During 1902 there was no special prevalence of the disease outside the gaol.

Note IV. Gaol population is about 400—500.

affected and the item is not on their dietary. The facts at disposal are not sufficient to adjudicate between coincidence and causative relation in the effect of the change from supersufficient to sufficient diet, they were however suggestive of the latter.

(2) *Racial Distribution on Christmas Island.*

The class distribution on Christmas Island has already been mentioned, but there is a further aspect of the distribution in respect to race, which deserves attention both in this locality and in the Malay States.

The population on Christmas Island consists of Europeans (about 12), with a few Eurasians, Chinese (about 500—600, mostly Cantonese and Khehs, with a few Hokkiens and Hylams), Malays (about 20), and Indians, Sikh police (about 12), and Tamil coolies (who at the time of my first stay on the island were nearly 40 in number); there were also a few units, such as Bengali and Cingalese.

As has already been noted, the

1. *Chinese* suffered severely from beriberi.
2. *Tamils* also suffered from an epidemic.
3. *Malays* only give record of about half-a-dozen cases, of which two were fatal in the period from April 1901 to March 1903. I am indebted to Dr Giddy for these and other records of the island.
4. *Europeans* and *Eurasians* apparently escaped¹.
5. *Sikhs* did not suffer.

The *Bengali* was stated to have been attacked, and the *Cingalese* had a severe attack.

¹ As a personal experience, I may note that after about a month on the island numbness down the front of both shins occurred, about 3 weeks later this had extended to sides of calves, with quite marked, though slight oedema; about 3 weeks later still the numbness had gone, the oedema lasted some time longer, and disappeared about the time when the knee-jerks, which had been markedly exaggerated, became more normal; on the supposition that the collective examination of the coolies, which was done on five consecutive evenings, had any connexion with this, the "period to discovery" (*i.e.* incubation period + days of disease which elapsed before recognition of symptoms) would have been between 19 and 24 days. About the 6th week a short run gave an increase in pulse-rate of from 80 to 152, whilst two controls (European and Tamil) gave respectively 90 to 120 and 78 to 138.

(3) *Racial Distribution in the Federated Malay States; with general notes on the habits of the various races, especially of the Chinese.*

In the Federated Malay States the *race distribution* is on the whole similar. Beriberi is unknown or practically so amongst the *Europeans*¹; it is not usual amongst the *Eurasians*.

I met with one case in an Eurasian of Dutch extraction and examined his wife and family, none of whom showed any suspicious sign, or admitted suspicious history.

Sikhs likewise escape the disease, both in their outside life and almost always when confined in gaols. Many of the native warders in the gaols are Sikh, and though exposed in day and night hours to hypothetical beriberic effluvia, they never contract the disease (the same is true of the Bengali and Sikh attendants at the Singapore Lunatic Asylum).

Sikhs are mostly employed as police, watchmen, and the like, there are also a few independent carters. Their food consists of wheaten flour, as chief staple, which they usually make up into a sort of damper or "chupati"; not much rice is taken and that generally as "canji," *i.e.* rice boiled in water to a thin porridge. Of animal food, pork, mutton, goat, and fresh fish; several men informed me that they do not like dried fish and practically never eat it. Vegetables and fruit complete the list. Tobacco is not used. Alcohol is often used to excess.

Pathans are mostly in similar circumstances as the Sikhs, they too do not appear to be liable to beriberi. I saw one case in gaol.

I have no exact notes on the habits of the Pathan, but in general he is less particular in his diet than the Sikh, for it is not regulated by the same religious scruples.

Tamils as has been pointed out are very rarely stricken with beriberi except in institutions.

¹ One casual observation may be made here since it is so strikingly in conformity with an observation I made at Parà (*Report of Yellow Fever Expedition to Parà*, p. 59, Liverpool, 1902). A well-to-do Chinaman told me that the Chinese did not care to stay more than two or three years in the hot moist climate of the Malayan Peninsula, and that they endeavoured to "cool down" in China at similar intervals. The ways of the Portuguese on the Amazon agree then with the ways of the Chinaman, and be it noted that a considerable proportion of the wealth in the Malayan Peninsula is in Chinese hands. The Briton stays out in these climates for much longer periods without a break, and I must admit to a sense of disappointment at the lack of enterprise and energy which appears to me to obtain in them. It seems that the "West Coast of Africa" is the only part of the equatorial belt (within 10° N. and S. of the equator) which we treat with a sage respect in this wise.

Tamil coolies come over to the Malay States in considerable numbers; they are a shifting population; immigration contractors are said to allow 25% women, in order to induce the men to stay. They are employed on agricultural estates, and in road-making, &c.; but very few are employed at the tin mines. They come to hospital with readiness, so that as Dr Braddon has pointed out their ailments are well represented. Their food is largely vegetarian; the rice which forms the main staple is husked in a particular manner, for the paddy is steamed before the husking; this leaves the thin pericarp and testa (*zilvervliesje* of the Dutch) attached to the grain; this kind of rice is however not universally used, for on the score of cheapness a certain number of Tamils buy the ordinary Rangoon or Siam rice, which is milled without the preliminary heating. Before cooking the rice, Tamils were observed to remove dust, etc., by winnowing, sometimes without washing in water at all. Braddon has sought to find the escape of Tamils from beriberi in their usual habit of eating this kind of rice; Vorderman and others in Java, on different grounds, have sought to show that the "*zilvervlies*" has a protective effect, especially in the polyneuritis of fowls.

Currystuffs (tamarind, coco-nut, chillies, etc.) form an important item in the diet, which frequently contains dried fish, but rarely meat. The Tamil is not at all particular about the water he drinks; he is much given to alcoholic excess. He also smokes tobacco and chews "betel" (fresh leaves of the betel pepper, with scrapings of the areca, or "Pinang" palm-nut and gambir). His personal cleanliness is considerable, in conjunction with which he oils his skin. He generally lives in small or tiny huts on the ground, or in long, wooden sheds divided into small cubicles. Naturally the above notes do not apply altogether to the better class.

Tamils do not associate much either with Malays or Chinese, for there is mutual racial contempt between them.

Sewage disposal is casual.

Malays are occasionally subject to beriberi in their home life, but owing to their want of appreciation of European medicine they do not frequently come to hospital or consult European doctors, so that it is not possible to gauge accurately how widely spread the disease may be amongst them. It may be noted that they have a considerable contempt for the Chinese, and so do not associate with them. Besides Peninsular Malays there are a number of immigrant Malayan races, as from Sumatra, Java, and Borneo.

The staple diet of the Malay consists of rice and fish, the latter being usually dried, with currystuffs, vegetables, etc. Fowls, goat-flesh and beef are also eaten, but no pork, on Mohammedan principles. Curry¹ takes some work to prepare and several Malays living *en garçon* have told me that they rarely made curry, the fish is then either fried in coco oil, or merely scorched or toasted over embers.

¹ The stimulating effect of curry may perhaps be beneficial. I noted that the beriberic Malays, that I saw, gave histories of not having had much curry. The other curry-eaters, Tamils, likewise escape outside, but not in gaol. Both are "betel" chewers.

Sewage disposal is casual.

The Malay is a strict teetotaller; he smokes tobacco in cigarette form and chews "betel." His house accommodation is generally small, either on the ground or raised on posts. A few renegade Malays take to opium, as also a few to drink.

Chinese may be classified in several ways for our purpose. It is amongst them that most of the beriberi occurs. Thus they may be divided into the *rich* and the *poor*, with an *intermediate group* (mostly artisans or small shopkeepers); it is amongst the poor that beriberi mainly occurs.

Again, they may be classified according to their length of residence into *Sinkhehs* (new-comers on one-year contracts from China, who are considered the chief sufferers from the disease), *Lowkhehs* or longer residents. Amongst the latter I have met with many cases of beriberi which gave a history of having been 10 and even 20 years in the States and often that they had never had it before. *Straits-born Chinese*¹: these are much fewer in numbers and by repute beriberi is practically unknown amongst them (I have asked several Chinamen to what cause they attribute the escape of Straits-born Chinese, and they always replied that they are more careful in regard to what they eat, which perhaps is doubtful in the case of the poorer ones). And lastly *Towkays* or wealthy Chinese, amongst whom beriberi seems to be unknown.

When classified by *race* the most prominent point that is to be noted is that the *Hylams* are not attacked at all commonly; the majority of the Hylams are employed as domestic servants, there are also some engaged in fishing on the coast. I only met with one case in a Hylam "boy"; his master became ill (aguc) and went away to recuperate; about 20 days after his departure the boy became ill with numbness and weakness, eventually he was unable to walk and had some attacks of dyspnoea, one of which was fatal. It may be added that three other servants were left in the house but none were affected so far as I could learn. Whether the absence of scraps from the master's table had anything to do with the illness it is of course impossible to say.

Anyhow the salient points in regard to Hylam "boys" are that they are in association with Europeans or better class Chinese, and have many potential possibilities in their dietary.

In race, the other Chinese are mainly *Cantonese*, *Khehs* and *Hokkiens*;

¹ The escape of Straits-born Chinese is very hard to explain, unless it is similar to the "escape" of natives in yellow fever zones; anyhow curry eating is not widely spread or constant enough to explain it.

but classified thus they do not give any sign of special distribution of the disease so far as I am aware.

Female Chinese. In regard to *sex*, there is but little to say; females are proportionately few to the males, but inasmuch as there are over 20,000 of them in the States (Census, 1901) there would be enough to yield cases of beriberi; but the Chinaman is shy, and generally speaking the illnesses of his women do not come within the ken of the white man; an exception must be made for a certain number, in that a considerable number of women live in brothels, and some of the keepers have a system of medical inspection. So far as could be gathered, beriberi is very far from common, notwithstanding that they live herded together; on the other hand their employment is fairly lucrative and they follow where money is plentiful; it may be supposed that they are not usually stinted in food.

Locality of Residence. A separation may be made by division into *town* and *out-station dwellers*, so far as the Malay States are concerned (*e.g.* not true of Singapore). It is most striking that beriberi is not rife in the towns; it is from among the mining camps that cases come, and ward after ward of beriberics may be examined without meeting with cases from the towns, all give a history of coming from some mining station.

It would appear that the history of many places is, that a settlement is started, soon after work has begun beriberi crops up, and increases to a dreadful extent; the communications and means of transport are improved, and beriberi ceases to be a terror. This may be applied to the States as a whole, where according to accounts the fatal importance of beriberi was much greater than it is nowadays. On a smaller scale the same may be said of Bentong (Pahang) which was ravaged, but now that better means of communication have been opened the disease is less; the same too may be said of Christmas Island. There is however at least one qualifying consideration, which is that in opening out new places the crowding and herding together may be excessive, the accommodation may be bad, the food may be deficient in quantity and in quality, but a *comparatively large number of Sinkhehs (new-comers to the country)* is introduced: the Sinkhehs both by repute and by apparent actuality are especially liable to contract the disease.

It may be noted here that all Sinkhehs pass a few days in depots in Singapore whilst their registration is being effected; and it is difficult not to believe, from an infective or contagious aspect of the disease, that Singapore is responsible for a more or less constant stream of

beriberi carriers¹, who afterwards may spread the disease locally. It is quite an interesting point that in the Malay States the disease should be so much associated with out-station life whilst in Singapore it is a town disease; especially when it is remembered that a majority of those that come up from the latter place to the former are distributed to the out-stations rather than to the towns.

Work and Trades. Another mode of division is by the nature of the work or trade; this is a large and not very fruitful mode. Two points only are mentioned here. One is that the cooks or those on the kitchen staff, even in gaols, habitually escape.

A somewhat interesting case on Christmas Island may be noted here; a cooly, who was a friend of the cooks, slept in their house but worked and ate with his fellow coolies; he became beriberic, none of the cooks did, the period covered being five months. The likelihood of an occasional nocturnal gambol in the other houses cannot be excluded.

The other is that of the coolies at the tin mines. The men spend all the day except for mealtimes in the open air; the "mining" consists in working alluvial deposits which is chiefly surface work; the men seem to be of cleanly personal habits and do much bathing. (Sinkhehs are sometimes driven regularly to bathe at 4 a.m. as a hardening process.) They may be crowded in numbers such as 60 to 80 in a single large shed, or three, four, and more may live together in smaller houses. Where accommodation is limited there may be two occupants to each of many of the bed spaces; on a single visit², some such houses I have seen looked very favourable for the dissemination of a contagious malady, but only one or two cases could be found, though a neighbouring more reasonably filled house abounded with beriberic cases. These "kongsi," as they are called, are built on the ground without flooring; the side walls are more or less permeable for air by gaps and interstices between the boards or palm leaves, with either or both of which the construction is made. The larger kongsi are divided up by partitions, which though not roof-high cause some stagnation of air. Bed platforms of planks some 3 feet above the floor are fixed across and along. The

¹ In the event of the importation of Chinese coolies to South Africa it might be well if the emigration agents avoided bringing the men through Singapore, or at any rate insisted on stringent measures being taken in respect to the condition and crowding of the transmigration depots in that town. The introduction of a number of beriberics amongst the African mines might prove as disastrous there as it appears to have been in the Malay Peninsula and on Christmas Island.

² Even repeated visits to a given locality will not help much in such cases, for the population may shift and become impossible to trace.

Chinese cooly almost invariably uses a mosquito net, and moreover seems to keep it in order. In a large kongsi, generally too in a small one, one corner is reserved as kitchen. The roof has a high pitch and is thatched with "atap," or other sort of palm thatch. With the reservation made above there is fairly free ventilation.

There is generally some accumulation of dust and rubbish about the floor, but hardly enough of such untidiness to merit the appellation filthy, which I have heard applied. Notwithstanding the amount of standing water about the kongsi the coolies seem to be remarkably free from malaria. Streams of water or pools from old diggings are near at hand for bathing; plenty of water is a necessity for washing out the tin sand, and up in the hills quite elaborate systems of conduits are to be seen to bring the water to the tin ore deposits.

The staple food of the Chinaman consists of rice, fish, pork, and vegetables. In the case of the Sinkheh and in the poorer districts, that is where worked-out or poor land is being "mined," the fish is practically always of the dried variety, and pork is generally given out once a month, rarely twice; the diet is supplemented with vegetables both fresh and preserved (salted, pickled or dried); the kapala or headman does not always get the best quality either of rice or of fish. In districts where there is more money to be obtained the worker is able to supplement the fare supplied to him. When working under contract to a kapala or when as in many places small sets of men agree to work together and share the results of their work every month, they live more or less independently and each may cook for himself or one may remain to take care of the house and see after the commissariat; this may be by rotation or one may be permanently the cook. In richer workings the men have pork every day as well as some dried fish, which is rather eaten in small quantities as a relish than as a real food: on festive or propitiatory occasions fowls, etc. are added to the menu. About the workings there is usually at least one patch of garden, where sweet potatoes and other vegetables are grown. From what I have seen in the Malay States the ordinary tale that a Chinaman will live and work on a "handful of rice" is by no means true. In one working I visited far away in the jungle the men were in the habit of getting three days' supply of pork and parboiling it at once in order to make it keep.

Many other foods might be mentioned in connexion with the diet of the Chinese in the Malay States, such as peas, beans, a sort of vermicelli, sauces, etc. Certain kinds of beans are made into "beancake," a sort of jellylike mass; beans are also allowed to sprout before they are cooked by boiling ("tow-gay"). The Chinaman is fond of sugar and sweet things.

In regard to cooking by the Chinese, the dried fish is usually fried in fat or oil directly or after washing with water; occasionally with certain kinds it may undergo a preliminary boiling in water, but this does not appear to be a common practice. In the Perak State gaol (Taiping) by routine the dried fish is always steamed before being fried: this was a difference between this and the Selangor gaol, which seemed to be a circumstance connecting the prevalence of beriberi

at the latter place and its absence at the former with the mode of cooking this article. Pork is boiled. Green vegetables are more or less fried in fat or oil. Rice is mainly cooked with the water which does not drain off after washing three times with water; in the form of "canji" or "bobor" it is boiled with water.

The Chinaman is very abstemious from alcoholic drinks; he chiefly drinks boiled water in the form of tea.

He smokes tobacco in pipe, cigarette or cheroot (some of this tobacco is stated to be arsenicated in the curing process, the smoke of Chinese tobacco usually has a peculiar, somewhat garlicky odour). Opium smoking is commonly practised, occasionally to excess; opium can also be obtained in "tabloid form" of Chinese manufacture; some of such preparations are vaunted remedies for curing the opium habit. A Chinese interpreter informed me that for a few cents hypodermic injections may be obtained in certain places in Pinang and Singapore.

Sewage disposal consists in collecting the material and using it in crude form on kitchen gardens. That this is not contributory to beriberi is shown by the fact that the Christmas Island coolies had practically no fresh vegetables, and also by the fact that the "refractory" races eat these vegetables also.

He does not chew betel. Only a few Chinamen who have long resided in the States, with whom may be included some of the Straits-born, eat curry.

Japanese. Quite a number of Japanese women and girls reside in the Malay States: they are employed in brothels. Their employment is lucrative, for they follow to districts where much money is being made; on the other hand they leave a district as soon as the tin ore becomes scanty. The number of Japanese in a place gives some criterion of the value of the deposits which are being worked. It may be inferred that their dietary is not stinted.

Whilst beriberi does occur amongst them, it appears to be quite uncommon, though Japan is quite one of the centres of beriberi and though they are associated with the Chinese in the Malay States. One girl with well marked beriberi was seen in hospital at Ipoh, the history given was suggestive of the disease having been acquired in Hongkong or in Singapore. Another patient, not beriberic, gave a history of having been laid up with beriberi in Hongkong. Three brothel mistresses of three, four and ten years' residence in the Malay States said respectively that they had met with one, one and three cases of "kak-kè" (beriberi) respectively during the time. Dr Travers informed me that though he had seen cases amongst these women, it was not common. It may be added that when ill they consult European doctors, so that if the disease were at all prevalent amongst them it would be known. Some enquiries concerning diet did not elicit much information. Whether their escape from beriberi is due to the relative infrequency of the disease amongst females, which is asserted by most authorities, but denied by Grimm, is a question which cannot be answered here.

(4) *Review of the Habits of the Natives in these Parts.*

In this short survey of the ways and habits of the different classes of the population in the regions visited, two main distinctions may be made according to diet; namely, the WHEAT eaters and the RICE eaters, that is to say those whose farinaceous mainstay consists respectively of these cereals. To the former belong the *Europeans*, the *Sikhs* and some *Eurasians*, and amongst them the incidence of beriberi is *extremely low*. To the latter belong the *Chinese*, the *Tamils*, the *Malays* and some *Eurasians*, and it is in and amongst these races that the incidence of beriberi is *high*; but even here the disease is not uniformly distributed, for whilst the Chinese and Malays are affected in their natural mode of life in the place, the Tamils practically only suffer when confined in gaols and the like. Though it is to be admitted that the Tamils, for the most part, consume a rice which is prepared in a different way to that which is eaten by the Chinese, the assumption of a rice theory to account for the disease does not remove the following obstacle, namely, that many of the Chinese likewise escape though constantly consuming the so-called dangerous kind of rice. Thus the town-dwellers and the better-off classes are not afflicted, whilst those who are in less good circumstances or in more inaccessible regions are not so lucky. (I only heard of one case in a "datoh" or leading *Malay*, but unfortunately I was not able to investigate it.)

(5) *Differences in the amount of Phosphorus in the Foods.*

When looked at from the broad point of view it is difficult, if not impossible, to renounce all idea that food and beriberi may be linked together somehow, and many of the published records are in favour of some connexion, though as a rule the writers are adherents of one or other theory which incriminates some particular article.

The records of the Pudu gaol show that the old theory of *nitrogen starvation* cannot be upheld, and the temporary lull in sickness in the endemic focus at Christmas Island during a period when insufficient nitrogen was being given is a further item against such a theory; though many of the coolies actually had beriberi at this time, it did not become severe enough to incapacitate them from work.

There is another constituent of food which has not, I think, been referred to in connexion with beriberi, but it may be observed that the

wheat eaters and the well-to-do obtain more *phosphorised* matters in their food than do the poorer rice eaters. The suggestion may be made that a sufficient amount of assimilable phosphorus in the diet has some power of fending off the essential beriberi poison or of protecting the nervous system from its action.

It is known that the addition of fats to the diet is not without importance in beriberi, but I have not been able to discover whether the presence of these bodies has any direct influence on the absorption of phosphorised compounds; it is however perhaps suggestive that the carnivorous animal passes ingested or injected inorganic phosphorus compounds through its system by the kidney, whilst the herbivorous animal passes it almost entirely by the faeces; the same is true of the glycerophosphoric acid (see Bergmann).

The statistics of the phosphorus content of different foodstuffs are mainly those in which the estimation was made by incineration, a method which would be calculated to leave much organically combined phosphorus out of account; possibly these are the more important, *e.g.* lecithins. I made a single estimation from some of the dried fish (one of the Scombridae) by oxidising with nitric and sulphuric acids, with addition of potassic chlorate (Halliburton); eventually iron was eliminated by means of citric acid and the phosphoric acid weighed as magnesian pyrophosphate; Mr Anderson, analyst to the Christmas Island Phosphate Company, gave me his kind help in this.

20.2 grams lost 7.8 grams on desiccation at 100° or 38.7% moisture, after treatment as above 0.3674 g. of $\text{Mg}_2\text{P}_2\text{O}_7$ were obtained, which gives a percentage of 1.15 of P_2O_5 in the moist fish freed from bones or 1.88% when desiccated. (Another sample of 55 grams gave a moisture percentage of 40.0.)

Though this is but a single analysis, I feel justified in recording it, as Katz has obtained comparable figures in his estimations of total phosphorus in various meats. Thus he gives

Dry pike flesh with fat	2.35 %	reckoned as	P_2O_5
Dry eel flesh " "	1.09 %	" "	" "
Dry pork with fat	1.79 %	" "	" "
Dry beef " "	1.60 %	" "	" "

These figures are naturally much higher than those obtained by the incineration method; the following of which may be quoted as showing considerable differences in the phosphorus contents of the various substances.

Thus the ash method gives about 0·8 % in wheat, 0·8—0·9 % in peas and beans against 0·19 % in rice, all reckoned as P_2O_5 ; also beef yields 0·285 %; mutton 0·425 %, and pork 0·16 % (it may be noted that the pork eaten by the Chinese is mainly fat). Probably no observer who has had to deal with beriberi cases will deny the good influence of adding peas, beans, milk, eggs and the like to the diet of patients.

Apart from the general deficiency of metabolism in beriberi, to which I have recently recalled attention (*Brit. Med. Journ.* Vol. I. 1904), it should be noted that the appetite in the disease may be very bad. Visits to beriberic wards at mealtimes always showed many unemptied plates. Mr Galloway of the Pudu gaol pointed out to me that as soon as matters mended in the epidemic of 1902 there was a marked diminution in the amount of waste rice which is disposed of day by day.

III. Experiments on Animals.

The following are notes of a number of experiments which I made in the hope of inducing beriberi in animals. Monkeys of the genus *Macacus*¹, small coconut monkeys or “brok” in Malay (*M. nemestrinus*), and the longtailed monkeys (*Macacus cynomolgus* and another species) or “kěra” in Malay, were used. A few rabbits and guinea-pigs were also tried. It must be admitted that at the present time we do not know that any of these animals are capable of contracting the human disease, beriberi; several authors have claimed to have reproduced the disease in animals but it appears that the cases claimed are merely of the nature of septic neuritis.

Recently Hamilton Wright has claimed to have induced beriberi in monkeys, but whilst the looseness of his report may be sufficient indication of the looseness of the observations upon which it is founded, it may be well to add a note concerning these monkeys. For instance there is no reason to suppose that his “monkey 27” was anything but a case of neuritis due to septic absorption from an old standing chronic deep ulcerated cut in its neck, which was caused by the wire collar whereby it was chained. Two monkeys from the same batch, from the same place, which were under my observation may be regarded as controls, especially one which became emaciated and weak through an ulcerated cut round the abdomen caused by a retaining band; owing to want of space this monkey had been chained outside a cage; it had been regarded as stock, but was killed at once when my attention was drawn to its condition; its liver was full of abscesses, but as it was not anticipated that there would be any special interest about it no cultures were made; the nerves,

¹ The anthropoid Gibbons would perhaps have been better for such purposes.

however, were prepared with Marchi's method and were found to be in an advanced state of degenerative change: this was most marked in the anterior tibial, but present in other limb nerves as well as in the phrenic. The second monkey had no external lesion, it became rather suddenly ill with marked weakness, the knee-jerks exaggerated and intensified response to blow on muscles. At the post-mortem the apices of both lungs were solid, with the exception of a cavitation of the left; very scanty tubercle bacilli and abundance of staphylococci were found; the nerves examined showed many extensively degenerated fibres. Neither of these monkeys had been exposed to any presumable beriberic influence. Both of these as others in the same batch were found to be infested with a malaria-like parasite in their red blood corpuscles, and the temperatures of five out of the batch were all very irregular, *e.g.* rising to 103°, 104° and the like (the statement in regard to "monkey 27," that its temperature did not exceed 100·5° till July 18th, is perhaps explicable by the desire to please, or by the gratitude of the small responsible native assistant for having escaped conviction on a charge of perjury, though he soon after was imprisoned for two years for theft and forgery¹).

Owing to these findings the nerves of every animal that came to post-mortem subsequently were examined, but it was only in these two obviously septic and possibly "malarial" cases, and the one noted in text, that degeneration was found.

It is also to be regretted that the suggestion of Drs Gimlette and Travers to expose animals to the atmosphere of the cells of the gaol was not arranged in a more satisfactory manner; thus the cells by direction were allowed to be in a nauseatingly filthy condition, well calculated to make any free living beast fresh from the jungle ill. But it would occupy too much space were I to go further into the statements recorded in this report.

As will be seen below only one monkey gave a suggestion of possibly artificially induced beriberi, and attempts to repeat the experiment proved without satisfactory result; although this monkey did not apparently suffer from any ordinary septic influence, in the light of the two controls mentioned on p. 128 I am not disposed to assert positively that it had a beriberic neuritis.

(1) *Feeding Experiments (Dried Fish and Rice) on Monkeys.*

First, having been impressed by the apparent connexion between dried fish and beriberi, some monkeys were given portions of dried fish; one control received nothing but boiled rice, and one monkey received fish and rice only, the remainder had fruit, &c. as well. None of these showed any really suspicious symptoms. The rice-and-fish monkey after three months on fish and three months on rice and fish died about a month after resumption of fruit diet; nothing was found to account for death, but its nerves were healthy.

¹ See *Malay Mail*, June 13, 1903.

The fish used included a sample which had been implicated on Christmas Island, as well as a number of samples which were bought in the open market and kept aerobically, anaerobically, or infected with the above-mentioned suspected fish.

So far as they go these experiments were negative.

(2) *Do Bed-bugs carry Beriberi?*

Two monkeys were subjected every few days to the bites of a number of bed-bugs which had been captured from time to time about the bed-boards occupied by beriberic patients at the Lunatic Asylum and General Hospital (gaol ward) at Kuala Lumpur.

These experiments proved negative. The bugs refused to bite a rabbit.

It may be noted that it was possible that the bed-bug might be a carrier of beriberi from some points of view; but whilst these insects are common in Tamil home quarters, during the gaol epidemic I failed to find any about the cells on two or three occasions. I did not succeed in finding any in the coolie houses on Christmas Island. In a house at Tras (Pahang) (*v. p.* 137) where a number of men had been affected with beriberi (*vide infra*) bugs were found abundantly in an old piece of sacking; some were collected for experimental application, but all died in transit from the breakage of a formalin bottle.

(3) *Effect of Injection of Serum of Beriberics into Animals.*

Serum. The fresh blood sera of persons dead of beriberi and of persons suffering from the disease were injected hypodermically in quantities of 10 to 30 c.c. into monkeys and in less quantity into a guinea-pig and rabbit; beyond a passing local oedema in the guinea-pig and a superficial necrosis of skin in the rabbit there was no result to record. This negative result agrees with those of other authors.

(4) *Administration of contents of Gastro-intestinal tract of Beriberics by the mouth to Monkeys.*

A series of monkeys was given by the mouth quantities of the gastro-intestinal contents obtained at fresh post-mortem examinations of beriberi cases. About 10 c.c. were swallowed or given by a catheter passed into the stomach. The doses were repeated once. The matters thus tested were the contents of the stomach, duodenum, jejunum, ileum and colon. In no case did any suggestive result accrue. Cultures on

agar plates were made at the same time: the small translucent colonies which were sometimes abundant all proved to be ordinary streptococci. Otherwise there is nothing worthy of record.

(5) *Administration of Dust to Monkeys from "infected" Localities.*

One monkey had its throat rubbed with dust obtained from crevices in a bed-board of a cell in Pudu gaol; two months later it weighed 1114 grams. At this time it unfortunately received an injection of 10 c.c. of beriberic serum¹. 113 days after the dust application (55 after injection) it was rather ill (weight 940 grms.), and had an erythematous patch in the groin and about the feet. Solution of sulphide of soda was applied but it proved caustic and caused a slight superficial ulceration two days later. The knee-jerks were markedly exaggerated. Two more days later the knee-jerks could hardly be elicited, there was no oedema. Next day it died, viz. 118 days after commencement. At autopsy the liver was very pale and fatty; there was very little fat about the body; the suprarenals were somewhat gelatinous in appearance (a condition I have noted at some post-mortem examinations of men dead of beriberi), and there was a small superficial ulcer in the left groin (from the sulphide). Otherwise the organs were merely anaemic. The limb nerves and the phrenic showed much degeneration by Marchi's method; whether this degeneration was due to beriberi is a question that cannot be answered.

Naturally I instituted other experiments with dust applications and also confined two monkeys in a packing-case with some mats which had been in use by coolies who had died of beriberi, but none of these gave definite results.

(6) *Throat to Throat Infection Experiments on Monkeys.*

Since the administration of the contents of the stomach and bowel had been negative, trial of application of mucus from the throat of a patient directly to the throat of a monkey was made.

A monkey was first swabbed from the throat of the above-mentioned monkey [Section III (5)]. Two months later its throat was swabbed with mucons matter from the throat of a beriberic case; two and a half months later it had somewhat exaggerated knee-jerks and the direct muscle reflex was well marked; but it was not killed for nerve examination, so that the result was not satisfactory.

The other monkeys did not show any symptoms.

¹ There was a lack of animals.

IV. Observations on Man.

(1) *Clinical observation of the Throats of Beriberic Patients.*

Clinical observation of the throats in two places so distant as Gopeng (Perak) and Christmas Island (more than 1000 miles apart) as well as in Kuala Lumpur seemed to indicate that in early cases and those which had recently come to hospital, there was a marked faucial redness. This condition was not associated with tenderness or swelling of the lymphatic glands.

Sterilised cottonwool swabs mounted on wires were smeared on the throats of a number of patients and then on the surface of agar medium in Petri dishes. After 24 hours, but better after 48 hours, remarkable numbers of small low translucent grayish colonies appeared on the plates; after 96 hours they measured only about 1 mm. in diameter. Under a low power of the microscope they showed a well-marked distinguishing feature in that there were tiny loops of projecting organisms around the periphery. Some plates were crowded with these "small looped" colonies and this almost or quite to the exclusion of other kinds of colonies. Morphologically, they assumed a somewhat streptococcal appearance in short chains, but there was a great tendency to the formation of involution forms, of a swollen irregular or rod-like character; no motility could be detected. The organisms retained the stain after treatment by the Gram-Weigert method and showed some capsular material. A considerable number of plate cultivations were made from gaol dust and dust obtained from suspected sleeping mats, but none of these revealed similar colonies. The same may be said of the cultures which were made from the intestinal tracts¹ of beriberic corpses.

It would appear that some constituent of the mucous material in the mouth is necessary for the development of the organisms described above (cp. *Bacillus influenzae*), for subcultures all failed to grow on being transplanted on to fresh agar or into broth. Original cultures which were brought back all died out on voyage.

The following are notes of the last 5 cases examined:

(1) Lim Chin gives two months' history; can just walk; knee-jerks absent; direct muscle-reflex markedly increased with fibrillary wheal. Fauces and pharynx very red.

¹ Dr Mott, I think, was the first to observe the reddened condition of the duodenum in acute cases. The redness may extend a considerable distance along the small gut; it would appear to be a secondary phenomenon.

Throat examination : *microscopically*, chiefly micrococci with some Gram-Weigert staining bacilli.

Plates from the throat :

24 hrs. Very abundant small translucent colonies.

48 hrs. Very marked abundance of small "looped" colonies.

96 hrs. On one plate only five, on second only one other than small translucent "looped" colonies.

(2) Leong Suk gives history of ten days ; knee-jerks absent ; direct muscle-reflex markedly increased ; can hardly walk. Throat reddened.

Plates from the throat :

24 hrs. Very abundant small translucent colonies with looped edges, with a few large white opaque colonies.

96 hrs. One streak consists entirely of numbers of small looped colonies.

(3) Theng Yoon gives history of 4 months and has been 13 years away from China. Knee-jerks absent ; direct muscle-reflex in leg rather lively, in arm markedly exaggerated ; slight oedema of shins.

Throat : *microscopically*, cocci, diplococci and a few rods.

Plates, 48 hrs, very abundant growth, nearly all small looped colonies.

(4) Vong Heng gives history of 2½ months' illness and has been 7 months away from China. Calves very flabby and tender ; knee-jerks absent ; no oedema ; cannot walk. Throat not reddened. *Microscopically* few cocci and few bacilli.

Plates, 48 hrs, not many colonies, but several of them are of the small looped variety.

(5) Lim Chee gives history of 2 months' illness and left China 22 years ago. Can walk in the "typical" beriberic manner ; knee-jerks absent ; no oedema. Throat slightly red ; *microscopically* many cocci with some club-shaped bacilli.

Plates, 48 hrs, abundant colonies, nearly all of the small looped variety.

The above are samples out of the examinations which were made. It was obviously important to see whether this type of organism was a common inhabitant of the throat, or whether it was solely to be found in connexion with beriberi. Unfortunately before proper control observations had been established the local heads of the Government became so discourteous that it was not possible to consent to remain working in their sphere, where one had to be more or less in the position of a guest of Government. Unluckily, too, there did not appear to be a favourable locality for prosecuting the matter at the time, so that I reluctantly returned home with this portion of the investigation uncompleted.

(2) *Notes on some clinical aspects of the disease.*

Knee-jerks. Too little notice is I think taken in most books of the preliminary exaggeration of the knee-jerk, which so commonly precedes the loss thereof. Whether the knee-jerk is ever lost without a previous

stage of increase I am not able to say, but observations on a number of patients showed that, given the increase, it may remain and never disappear before the patient's apparent recovery, or it may slowly decrease to absolute loss, or it may disappear rapidly in a few days, giving way to complete absence. Recovery of the knee-jerk after loss seems to be a very slow process, but that it is eventually regained there can be no doubt if the history of having had the disease in several prisoners and others is to be believed. Increased response to a blow on the muscles seems to obtain generally after the diminution of knee-jerk has occurred.

Tenderness of calf muscles. It appears that rather too much prominence has been laid upon this symptom, so far as my experience goes. For though I have squeezed the calves of many hundred beriberics, I cannot remember having caused flinching or wincing in more than half-a-dozen cases. Nor taking cases in all stages of the disease do they often complain of pain having been caused.

Variations in prominence of symptoms. Several authors have already insisted upon the variable prominence of certain symptoms in different epidemics, and this is a matter which my own observation confirms. But I do not think with some of these writers that this is to be regarded as indicative of a different breed of beriberic noxa. Thus the last little epidemic that I saw on Christmas Island was characterized by very marked affection of the heart, with very slight or absent oedema and much exaggeration of knee-jerks; still at the same time there were a few cases of severe "wet" beriberi. Indeed wherever there were sufficient cases, one could see examples of all the different varieties as a fairly complete clinical picture of a single disease.

Hoarseness of the voice is a symptom which seems to be more frequent in some epidemics than in others.

At my first visit to Christmas Island there was a great deal of stomatitis (swollen sore gums), but judging from the complete absence of this at a later period and in other places I hardly think that it can have any causal connexion with beriberi.

V. Remarks on the Epidemiology of Beriberi.

It would seem from the literature of beriberi that none of the theories which have been advanced will explain satisfactorily all the accounts which have been given. Often these accounts are wanting in sufficient completeness for pronouncing an opinion. An instance of the

unsatisfactory nature of the literature may be cited in the oft-quoted outbreak in and about New Caledonia, the account of which has led to its being used to support the dried fish theory, the rice theory, the emanation theory, and also the infective theory. It is clear that if one of these theories be true the others must be essentially wrong.

During the eighteen months that I spent in the Malayan region I made a considerable number of visits and inspections with the specific object of obtaining information concerning beriberi. I feel, on looking through the notes made at the time, that the greater the amount of facts that I was able to discover, the less could the possibility of some direct or indirect contact be eliminated. Naturally much has to be discounted from the accounts given by natives however well meaning; but occasionally I met, almost accidentally, with information from independent sources which tended to show that possibility of contact had occurred through the introduction of persons suffering from beriberi.

Scheube considers that the contagiousness of beriberi can be excluded, and I have met with cases where contact was present and the disease was not acquired (see p. 119, case of Eurasian and wife, and p. 123, case, living with cooks on Christmas Island). But on any theory such cases must be explained by the mysterious variations in susceptibility towards the noxa of beriberi, whatever that may prove to be. It seems probable that the food, or absence of a sufficiency of certain requisites in the food, bears some intimate relationship with the proneness to acquire the disease.

Further, on the assumption that beriberi is due to an infection of the throat region, the preexisting bacterial flora will have an effect upon the variability of and poison production by the effective microorganism. In the growth of the diphtheria bacillus we know that the presence of certain sugars in the medium, while leading to the production of acid, may more or less completely inhibit the production of toxin. Just as it is possible that the presence of other organisms capable of producing acid from the pabula in the mouth may alter the nature of the attack, so in beriberi it may be that some more or less fortuitous condition of flora or constitution of food remnants may have their due effect. There is also the question of an acquisition of immunity in beriberi (*e.g.* by slight attacks), but whilst there seem to be indications that such may exist actually, it will be better to defer consideration of this point until the presence of specific "antibodies" can be demonstrated.

(1) Outbreaks in Isolated Places.

That the cause of beriberi is to be found in and about the person or the personal apparel and personal furniture is suggested by the way in which the disease has disappeared from regions with the removal of the persons who brought it. Just as in Fiji the disease ceased when the Japanese left, so on Christmas Island a party of Klings who lived together in certain houses (one a new house) far removed from the Chinese quarters were severely affected during my stay there; but when they and their small amount of baggage left the island, there was no further trouble in that house during the next 12 months.

That the noxa of beriberi is carried in close relation with the person is also suggested by an outbreak which occurred amongst a party of Chinese carpenters whom I saw and examined on their return voyage to Singapore. They had been for a few months on the previously uninhabited Direction Island of the Cocos-Keeling group.

Out of the 12 men 2 died (one was seen to die, and undoubtedly did so of beriberi), 3 had severe beriberi, 2 had slight beriberi, 3 seemed quite normal, 2 were not examined.

It seems clear that the beriberi noxa must have been taken down to the island somehow in some vehicle or other. The vehicles which are apparently possible are (1) the one or more of the persons themselves, (2) some part of the scanty baggage with which this class travel (sleeping mat, blanket, a change or so of clothes and box of tools), (3) the food taken. Though I believe that food or some constituent of food may have a distinct influence upon the susceptibility towards the disease, since I have not investigated any outbreak in which every possible factor save food can be definitely excluded, I am inclined to disbelieve that the food can have done the mischief. (4) The men may have acquired the disease from the sailors or the vessel upon which they reached the island (the voyage would have been of about 5 days' duration); upon this I am unable to give any opinion as I have no knowledge of the vessel. Unfortunately I have no reliable date to fix the first commencement of the outbreak, but since the men were on the island from Sept. 1901 to Jan. 6th, 1902, it is possible that the disease was spread on the island. Perhaps one of the men who was found to be normal on the return voyage was the refractory or immunised carrier of the noxa.

The following case of the occurrence of beriberi may be mentioned here, since personal contact appears to have shown some influence.

A party of picked Malays (South Borneo) was brought over to work in the jungle in Pahang. The men suffered severely from beriberi soon afterwards. Their employer, Mr Ponsford, was certain that they had acquired the disease from eating dried fish away in the jungle. For a short while they were accommodated in a house in the village of Tras, which had previously been occupied by Bengali bakers. With one exception they remained but a short time in this house. The exception was a man who injured his foot by a falling log, he was laid up and remained in the house; of the whole party *he was the most severely affected with beriberi*. There could be no doubt about the symptoms in any of the men. It is, then, very improbable that the other men acquired the disease in the jungle, in a manner independent of their injured mate. It may be concluded that all caught the disease in the house. The presence of abundant bed-bugs has been mentioned on p. 130. It happened that I met a Malay "Haji" several miles away through the jungle, who was suffering from severe beriberi, accompanied by dyspnoeal attacks. He gave a most circumstantial account of having been in the habit of sleeping with the South Borneo men in the house. He obtained his meals at the house of the local "Datoh" (district head Malay). He also said that another man suffering with the same malady had been of the company. There was no reason to suppose that he was not telling the truth, his statement was voluntary, and he had no reason to fear. I carefully abstained from any leading questions. The house was an old wooden shanty on the ground, dark inside, but with a fair amount of ventilation; the weather was wet but the interior seemed to be dry.

This case is illustrative of some others, which might be quoted as instances where though it might be supposed that the disease had been derived from the infected condition of a house, yet, even so, the possibility of actual contact or close neighbourhood of persons suffering from beriberi could be entirely excluded.

(2) *Alleged House and Ship Infection.*

The question of infection of houses and ships has been given some prominence in the literature, thus Rees speaks of certain ships in a fleet as "beriberi ships," and he draws attention to the continuance of the disease on these ships. If, however, his tabulated statement be examined, it will be seen that the so-called infection of the ship was not continuous through the history given, but that on a given ship the disease disappeared to reappear again some years later. To such interruptions no attention was drawn and no explanation was offered, but it is not inconceivable that the disease was not directly acquired from the ship itself, but from some item of the crew; and then no difficulty is to be found in understanding the circumstances. It must be remembered that it is not always or only those suffering from pronounced infective disease of any sort that are necessarily the most

dangerous spreaders of the disease. Not infrequently it may happen that the unrecognised spreader does incalculably more harm. But in beriberi, as yet, there is no established criterion of recognition such as we have in the case of the diphtheria bacillus.

Another point to which reference may be made is the poor result which is obtained by house disinfection; thus the curves given by Pekelharing and Winkler do not give a noted effect of sublimate disinfection. This was very strikingly the case on Christmas Island, for during a period of about a year the houses (*i.e.* floor and bed platform) were swabbed out once a week with 1:4000 corrosive sublimate, later this was done once a fortnight, since the men complained of the dampness, which together with personal observation of the process shows that disinfection was fairly thoroughly carried out. Now this disinfection was only in force for the houses occupied by the coolies; the precisely similar houses occupied by the artisans *were not interfered with*. It has already been mentioned that the artisans practically escaped the disease whilst it still continued to ravage the coolies. It must be clear that if the causative agent lurks in the sleeping mats, clothes &c., or actually about the persons of the coolies, the house disinfection is not calculated to be of much service.

(3) *Alleged Importance of Removal of Patients from a Locality.*

Many writers tend to lay great stress upon the importance of removing patients from the locality in which they were taken ill. Whilst I do not desire to combat the belief that a change of air and still more of food is beneficial, yet it seems to me that removal does not benefit the patient suffering from beriberi to a greater extent than the patient suffering from many other diseases. It need hardly be asserted that with malaria, and almost any other infective as well as some noninfective diseases, removal does good which can hardly be obtained by any other means. Beriberic patients can and do get well locally; and though they stay in or about a focus of the disease they do not necessarily suffer thereby to the extent which might be imagined. In my own case, if indeed the condition, which did not consist entirely of subjective symptoms, was really of the nature of beriberi, I may mention that I continued to occupy the same rooms, and very damp, nay moist were they, where if some miasm inspired with each breath had been the cause of the complaint I should hardly have survived to tell the tale.

Again, the cooly who is discharged from hospital is not often really

cured but rather merely relieved, yet for instance on Christmas Island though he returns to his old quarters, to his old work and companions, yet he does not as a rule become progressively worse, though in cases he may again seek hospital care.

Dr Travers called my attention to an interesting point in that, during the extensive outbreak in 1902 at the Pudu gaol, owing to the large number affected, all could not be given accommodation at the hospital within the gaol compound, which indeed was much overcrowded with patients at times. A number of the patients were sent more than a mile away to the district hospital. A considerable amount of selection had to be made of the cases to which hospital accommodation could be given, so that it was only the more serious cases that actually were admitted or admitted and transferred.

As shown by the deathrate the patients did much better within the precincts of the gaol than they did away at the district hospital.

It is striking too, in the case of some of the outlying district hospitals, in the Malay States, how different the beriberi deathrates may be. Certain of these hospitals are in charge of native dressers; but so far as I have seen, although there may be some erroneous diagnoses, and some deaths may be due to intercurrent circumstances, yet these seem to be comparatively few. Particularly worth mentioning is the case of Jugra hospital, in which, as Dr Watson pointed out to me, the beriberic death-rate was very high, whilst the place itself hardly contributes to the beriberi cases; these are brought from a place many miles away.

Table to show difference of Deathrate from Beriberi in various Hospitals in Selangor State.

(The figures are for the first half year of 1902.)

	Cases	Deaths	Death rate %
Kajang	103	nil	0
Kuala Kubu	69	19	28·8
Serendah	113	6	5·3
Kuala Langat (Jugra)	10	6	60·0
Kuala Selangoi	nil	nil	—

Note. For these figures I am indebted to Dr Travers. We paid a special visit to Kajang to look up records and examine cases for control of current diagnoses.

Table to show high mortality from Beriberi in Kuala Langat (Jugra) Hospital.

(The figures are for 1902 and are analysed to show length of stay in hospital.)

No.	Patient's statement of duration of disease on admission	Where acquired	Date of admission	Date of death	No. of days in Hospital
1	45 days	Sepang	12.1.02	21.1.02	9
2	20 "	"	16.1.02	17.1.02	1
3	15 "	"	22.1.02	14.2.02	23
4	10 months	"	22.1.02	1.3.02	38
5	25 days	"	28.1.02	18.2.02	21
6	10 "	"	28.1.02	—	120*
7	30 "	"	11.2.02	—	90*
8	30 "	"	26.2.02	—	13
9	30 "	Jugra	4.3.02	—	7
10	3 months	Sepang	20.3.02	3.4.02	14
11	16 days	"	8.10.02	1.11.02	24
12	2 months	"	14.10.02	24.11.02	31
13	1 month	"	12.11.02	22.11.02	10
14	3 months	"	10.12.02	29.12.02	19
Total cases admitted ...			14		
Total deaths ...			10		
Mortality ...			71 %.		

* Approximately.

Notc. I am indebted to Dr Watson for these figures. He points out that all the cases except one were removed about 40 miles from the place where the disease was contracted. It will be observed that many of the cases were not fatal very soon after admission. I made other similar analyses of records but the one given will suffice to illustrate the point.

(4) *Length of Exposure of Persons before Acquisition of Beriberi.*

Some writers have insisted that there must be a prolonged exposure (*e.g.* years) before the disease is contracted. Observations on the incubation period are difficult inasmuch as the time of real onset of the disease is so ill-marked¹.

On Christmas Island I examined a number of beriberics who certainly had not been more than three months on the island. The men had passed a routine medical examination in Singapore which was designed to detect and exclude beriberics before they are shipped to the island. A batch of men who had been 16 days on the island was examined by me on the 9th of December, and found healthy except two with slightly

¹ The same may be said of the conclusion of the disease with recovery, so that such terms as "beriberic residual paralysis" or "post-beriberic paralysis" are at present without meaning.

increased knee-jerk. On the 22nd of January one of these men died of beriberi. On February 4th, of nine of these men who were gathered together for examination, only two could be passed as free from suspicion of beriberi. Supposing the fatal case was not incubating the disease on his arrival he passed from inception through incubation to the fatal ending in less than 70 days. These men were not "Sinkhehs" (see p. 121)

The following figures show the length of residence of one hundred consecutive fatal cases of beriberi on the island. I am indebted for these figures to the District Officer and to Dr Giddy.

Length of residence	No. of deaths	
2 months ...	11	
3 " 	21	
4 " 	6	
5 " 	12	
6 " 	7	Total up to 6 months, 57
7 " 	4	Total 6—12 months, 21
8 " 	2	
9 " 	3	
10 " 	2	
11 " 	8	
12 " 	2	
1—1½ year ...	20	
1½—2 years ...	1	
over 2 " 	1	

These figures tend to show that the greatest beriberi mortality occurs during the first 6 months of residence. It might be expected however that the numbers for the later months should be larger by virtue of invalids who had been sent back to Singapore and who might otherwise have added to the deathroll. But the contract period of time is twelve months, and comparatively few are thus disposed of, so far as I was able to learn, before their contracts mature.

The shortest period of residence on Christmas Island with a fatal termination through uncomplicated beriberi that I met with was in a cooly in Government employ (who had not been passed as medically fit before coming and may have been already suffering when he arrived). Signs of the disease were already well marked on the 16th day and he died on the 38th day after arrival.

(5) *Seasonal Variations in Prevalence.*

The places which I have visited, all within the equatorial zone, do not appear to show that there is any definite seasonal prevalence of

beriberi in this fairly uniform warm, damp climate. Dr Ellis has shown by his curves that the number of cases may reach its maximum in almost any month in the lunatic asylum at Singapore. (Compare table of admissions to Pudu gaol on p. 117.) On Christmas Island it had been supposed that the disease prevailed in the wetter months, but a very distinct outbreak occurred within what were the driest consecutive three months that had been recorded. In the converse sense, at the end of another small epidemic, the rainfall increased as beriberi diminished.

One fact which probably has no meteorological significance owing to the distances of the several places, is that in and about January, 1903, there was an almost simultaneous "disappearance" of beriberi in the Pudu gaol at Kuala Lumpur, in the gaol and lunatic asylum at Singapore, and on Christmas Island. It is hardly possible to say whether this could be more than a mere coincidence.

VI. Current theories of the Epidemiology of Beriberi.

(1) Food.

i. *Physiological.*

Diet as such, or *physiological* or *specific starvation*, has already been dealt with sufficiently in the foregoing pages. This aspect of diet has not always been clearly separated from the possible effects of unsound food.

ii. *Unsound Food.*

(a) *Exclusion of all articles except Rice and Dried Fish.*

Unsound food considered as a cause of beriberi has had many supporters. Looking at the racial distribution of the disease in the regions visited, it may justly be argued that the kind of food which forms the vehicle of beriberi must be one common to all the affected races. This practically excludes all foods with the exception of dried fish and rice. The Malays do not eat pork, though the Chinese do so. The Chinese do not eat curry. The other races do not eat Chinese sauces or preserved vegetables. Fresh fish, fresh vegetables and fruit, can likewise be excluded, because the Chinese coolies on Christmas Island did not obtain any of these articles except on quite isolated occasions.

(b) Rice.

The rice is practically all imported either from Siam or Burmah; it is milled without the preliminary steaming process already mentioned (see p. 120, Tamils). So far as I was able to learn the rice is distributed and consumed almost as fast as it is imported, so that it is not stored long locally. It is forwarded by merchants in Pinang and Singapore. Many enquiries were made by me to ascertain whether any additions are made to enhance its keeping qualities. Mr Cowan, Protector of Chinese, Perak, kindly gave me much help in this connexion. It appears that lime is often added in the husking process, partly to help the decortication, and partly to whiten the grain. We could not get any evidence that arsenic is purposefully added, and the evidence of the report on the samples of dust from the rice which I submitted to the Royal Commission on Arsenical Poisoning, show that it can hardly be supposed that arsenic had been added, inasmuch as the findings were extremely minute. Another point is that many of the bad samples of rice which I have inspected were full of weevils, so that even if arsenic had been added with intention, not enough had been put to prevent the development of these destructive agents.

When the distribution of the disease throughout the world is included, rice has also to be put on one side; thus in the outbreak at Richmond Asylum, Dublin, as on many Norwegian ships, it is stated that no rice had been consumed. It would be necessary therefore to bring such outbreaks into line with the rice theory either by supposing that it was not the same disease, or else that other cereals may be similarly infected with the causative agent; that is to say we reach the region of pure speculation without direct facts in support.

Two points which impressed me with regard to rice were that on *Christmas Island* all the rice-eaters obtained rice of the same quality from the same store (with a temporary exception of some men in Government employ). Yet, as has already been stated, it was the cooly who suffered. During one of my visits to the island, there was a severe outbreak of the disease amongst a party of Tamils; this was not coincident with any recrudescence amongst the Chinese coolies who lived about a mile away. These Tamils ate in company with the Malays, the accessory foods were dealt with by each but *the rice was taken actually out of the same pot of cooked rice*. Why then should not the Malays also have suffered if the rice were at fault? Again, in the *Pudu gaol*, patients in the gaol hospital were recovering, whilst about

the cells or work-places their mates were being invalidated day by day. *They ate of the same rice, which was all cooked together.*

Whilst no doubt mouldy rice has often been consumed in beriberi epidemics, this does not appear to be a constant circumstance. Since some writers advocate this idea, and compare beriberi with pellagra, ergotism, lathyrism, &c., it may be worth while pointing out that these diseases are accompanied by severe central as well as peripheral changes in the nervous system; whilst such are absent in beriberi.

(c) *Dried Fish.*

Dried fish of one sort or another is on the whole a more widely spread foodstuff than rice, and it appears to fit in as a food origin of beriberi in a more worldwide manner than rice. It is a staple of diet throughout the East, it is eaten on the Amazon, on Norwegian ships, &c. &c. Curiously enough the Dublin outbreak was preceded by the introduction of dried ling as a novelty on the diet sheet, but it is stated that those who suffered particularly did not eat any of it, so also in Pudu gaol many short-sentenced prisoners were affected yet dried fish is not in their fare.

Up in the hills in Pahang, I found that the Chinese, who were comparatively free from beriberi, were eating expensive tinned fish, as they said that the ordinary dried fish went bad. The Malays, who ate ordinary dried fish ("ikan kring") suffered much more from beriberi, so I was told, and certainly I saw more cases of beriberi amongst them than amongst the Chinese. I may add that I spent a week walking about the jungle from one working to another: men we met were examined and catechised on the spot; others were examined in their houses, or huts which were mere frames enclosed with atap palm thatch.

A good deal of the fish is preserved in fishing villages along the coast of the peninsula, much is also imported. As seen in some fishing villages the process of preserving merely consists in sun-drying, the larger fishes having been split open, the smaller not; they may also be parboiled by dousing in a large pot of boiling water before they are exposed on bamboo frames to the sun. When dry they are either done up in bundles or packed in tubs with coarse salt. On the whole the dried varieties are less sodden and decomposed than the salted ones.

The proximity of stores of dried fish is apparent by the smell. The consumer and small restaurant keeper often exposes his supply of fish to the sun for further drying, and when it is thus well dried it hardly

has any smell. Fish as obtained wholesale from the merchant, however, is often mushy and offensive. One kind of bacterial growth occasionally gives trouble and is mentioned by Vorderman, it consists in the formation of a brickred film on the surface of the fish. Dr M. J. Wright, State Surgeon, Perak, kindly obtained some of this reddened fish for me. The chromogenic bacteria found in it were a pink-red *Sarcina* and a brilliant yellow bacillus. Another sort of fish, stated to come from China (somewhat like our red mullet in size and colour), was found to be affected with a yellow bacillus which apparently tends to grow or produce its colour along the nerves, thus the lateral line stands out as a lemon-yellow streak. The liability of dried fish to certain bacterial infections was of interest from the possible origin of beriberi from this cause. It may be noted that the merchant from whom the red infected fish was obtained had given up trading in it on account of the way in which it went bad in his store (which included a privy). It has already been pointed out, however, that there are reasons for exculpating this article in beriberi.

(2) ARSENIC.

Associated with food theories is the suggestion that *arsenic* is the cause of beriberi. Manson has pointed out that clinically beriberi and chronic arsenical poisoning appear to be distinct from one another. It has also been suggested that some other mineral substance or inorganic poison may be the cause, but Manson has, I think, rightly insisted that the evidence of power of multiplication of the cause of beriberi is strong and controverts the probability of any inorganic source. Ross, who has advocated an arsenical origin to beriberi, has recorded the finding of arsenic in the hair of beriberic patients; but the results of the analyses he gives are really opposed to his hypothesis in as much as only traces of the metal were discovered.

I made some attempt to determine to what extent arsenic is used by the Chinese. Mr Cowan very kindly gave me much help in gathering information on the question. It appears that "white arsenic" can be bought for about 20 cents a kati (say 5 pence for 21 oz.), and that it is used for the destruction of the white ant. In a list of Chinese drugs "arsenic," "white arsenic," and "red arsenic" were included; we made enquiries at two druggist's shops, but we could not get any admission that any of these were given as medicine. So far as we were able to determine it would only be as an impurity of other drugs that arsenic would be taken in medicine.

I may here interpolate a case of "burning palms and soles," the only one I met with in a Chinaman. Though he was somewhat like a beriberic in other symptoms it seemed possible that he was an arsenical, or a combined arsenical and beriberic case. Samples of his hair were sent to the Royal Commission, but they were not fortunate enough to reach the analyst. He gave a very distinct history of having been for six months under a Chinese doctor whose medicines caused a tendency to diarrhoea and intense griping; the burning in the palms and soles began three months after this treatment began. It may be added that several other cases of "*burning feet*," all in Indians, were seen, but it was not worth sending samples of their hair for they were all having arsenic administered therapeutically; the results did not seem to lead to cures or improvement. Enquiries as to cosmetic preparations failed to elicit any information as to the addition of arsenic.

Out of many hundred beriberics that passed through my hands I only once saw a case of *herpes*; this I think may fairly be put down as incidental.

I went to some trouble to determine whether *pigmentation* occurs in beriberi. The Chinamen vary very much in depth of colour—a certain small proportion are distinctly and markedly bronzed, and some of these individuals may have an arsenical coloration (see analysis of tow chang, p. 147). Personally I believe that the beriberic has a rather greater tendency to a deepening of colour on exposed parts, especially the legs; this may be due to the vascular changes which give rise to the oedema.

Enquiry of several "towkays" led to the presumption that arsenic is not intentionally added to rice for preservative or other purposes; quicklime is sometimes used in husking (see above, p. 143).

In order to see whether arsenic were added to rice, quantities of rice which was being consumed in Pudu gaol were sifted through fine wire-gauze sieves; the resulting dust was tested by the analyst of the Royal Commission (see Report, Appendix 31, p. 341).

Sample A was taken during a lull in the beriberi, July 28th, 1902. Amount of rice sifted = 200 lb., or about $\frac{1}{3}$ of a day's supply: total dust = 46.9 grms.; amount of arsenious acid = 3.2 parts per million of dust or $\frac{1}{40}$ grain per pound of the dust.

Sample B similar, but taken during height of fresh outburst of beriberi: total dust = 65.3 grms.; amount of arsenious acid = 2.0 per million or $\frac{1}{70}$ grain per lb. of the dust.

Sample C. Dust (Jan. 8, 1903) from whole week's supply of rice, i.e. about 4500 lb.; arsenious acid found = 5.0 per million¹.

Sample D similar to *C*, collected Jan. 19th; arsenious acid found = 5.8 per million¹.

¹ Total weight of dust not recorded.

In the appendix of the Report of the Royal Commission it will be seen that several samples of whole rice were submitted to analysis with the result that traces of arsenic were found in some samples and none at all in others.

In regard to fish (Norwegian cod and "Bombay duck") small quantities of arsenic were found by the Commission: since these did not exceed $\frac{1}{80}$ grain per lb., and if the eastern native's diet may be compared only about $\frac{1}{4}$ lb. of fish is taken a day, on this estimate there would not be a dangerous quantity of arsenic in his diet.

I made a few examinations of Chinese and Malay dried and salted fishes and of the salt with which the latter were covered and also of one sample of the phosphate rock, which is worked on Christmas Island. Owing to the acid having become arsenicated from the bottles and the Marsh apparatus which was sent out having been defective and also broken on the passage, measured quantities of hydrochloric acid were boiled for some hours with successive pieces of pure copper foil until little or no visible stain was caused; the matters to be tested were then introduced. Finally a drop of diluted Fowler's solution was added to see that a marked stain was capable of being produced and compared with the test piece of copper. Seeing that the results obtained from the objects mentioned were all negative, I think that they may be fairly considered to show that arsenic was not present in dangerous amounts. Some hair and nails were also examined, but the results were not sufficiently reassuring for inclusion here.

The Royal Commission kindly undertook the analysis of a number of hair and nail samples which were sent. In Appendix 31, page 344,

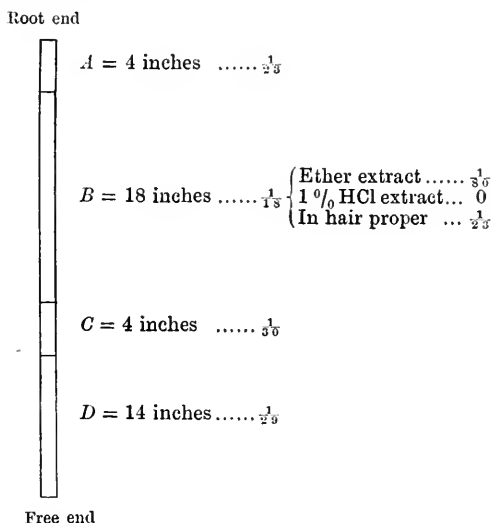


Diagram of "tow chang" to show distribution of Arsenic.

As₂O₃ in grains per lb. (found by Mr McGowan, Analyst to the Royal Commission).

of the Report of the Royal Commission on Arsenical Poisoning will be found the analytical record of two "tow changs" or pigtails from Chinamen recently dead of quite recent acute beriberi; these were examined in sections, of which the one contained $\frac{1}{15}$ and $\frac{1}{16}$ grain arsenious acid per lb. and the other contained up to an $\frac{1}{18}$ grain per lb. (see diagram). The arsenic, though in notable quantity, from being distributed all along the hair shows that the patients must have been in the habit of taking arsenic somehow for a long period. If an allowance of $\frac{1}{4}$ inch growth per week be made, *i.e.* very much in excess of the probable real rate of growth, the end of one "tow chang," which was about 26 inches long, would have been some two years old. The quantities stand out in contrast to Ross's own cases and also some other beriberi cases which were tested for the Commission. We can say then that though it is clear that arsenic must be partaken of by many beriberics (as also by normal individuals), the analyses which have been made are against the supposition that there is a connexion between these two clinically more or less distinct conditions.

In the same report is included an observation of Dr McClosky of Kuala Lumpur upon the therapeutical effect of arsenic given to beriberi patients. In his wards at the District Hospital, cases without selection of any kind were put alternately on doses of Liquor Hydrargyri Perchloridi, Liquor Arsenicalis and Mistura Chlorig (the latter I should mention consisted of Burney Yeo's mixture [see Squire's *Companion to the Brit. Pharm.*], without quinine: this I suggested as worth a trial, and, although not curative, it has been extensively used, since it gives as good results as any other medicine¹). The Liquor Arsenicalis was

¹ I may add that a small experiment to see whether Chlorine mixture would have a prophylactic effect, was made on the hypothesis that beriberi might be due to some ingested poison which could be destroyed by the oxidising action of the chlorine upon it. One ounce of the mixture was given three times a day to each of a party of 25 prisoners; a similar party of 25 men engaged at the same work (rotan shed) was examined to serve as control. The experiment was continued for 5 fortnights, during which time 14 admissions and 3 readmissions occurred amongst the chlorine gang against 12 admissions and 4 readmissions to hospital amongst the control gang. Whilst the result so far as protection was concerned proved negative, at the end of the period, judging by physical examination, the men of the chlorine gang appeared to be rather sounder than the controls, but the numbers are too small to prove such a point. I may note here, that it was intended to try the effect of adding phosphate of soda to the rice consumed by the prisoners; this however was not carried out as the disease ceased spontaneously in the gaol before the necessary salt arrived. Grijns records some negative therapeutical experiments on *Polynneuritis gal-linarum*. On my advice the same substance was to be tried in treatment of cases as a laxative instead of the usual sulphate of magnesia on Christmas Island, but reports thereon have not reached me. One other therapeutical experiment was tried, *viz.* to see whether the administration of carbonate of ammonia would increase the urea excretion or otherwise act beneficially; the patient was a fractious old man and the experiment was abortive.

begun with small doses and then pushed up to doses of 10 minims three times a day unless contraindications arose. Whilst it is true that the results were so far from favourable that the designed completion of 50 cases was not carried out, yet a few of the patients who recovered had taken heroic quantities of the acid. This being so, we have some therapeutical evidence against there being any direct connexion between arsenic and beriberi, as well as the clinical and the toxicological indications.

(3) MOSQUITOS.

That mosquitos have nothing to do with the spread of beriberi is probable. Three species of mosquitos were obtained on Christmas Island; these have been identified by Mr Theobald as *Stegomyia scutellaris*, *Culex fatigans* and *Culex alis* (nov. sp.). The former are solely day biters, they are abundant about the European and Malay quarters and some parts of the jungle but very scanty or absent about the Chinese coolie lines. The second were very scanty and only two specimens were taken, it appeared likely that they had been imported with a quantity of coconuts from the Cocos Islands. A mosquito net was unnecessary during the night, but most people used them, as the *Stegomyia* began biting at dawn.

In the outbreak amongst the Tamils to which reference already has been made there were some other Tamils and also Sikhs in houses interposed between the houses in which the cases occurred. If the disease were mosquito-borne it is difficult to account for the want of spread of the disease amongst these people.

Again, many of the outbreaks on board ship seem to be quite incompatible with a spread by means of the mosquito.

(4) COCKROACHES.

It has been suggested that cockroaches have some power of spreading beriberi. My own observations do not show that they were particularly common about the infected centres, which were visited. I only found a single one when rummaging after dust etc. at the Pudu gaol. Whilst it is possible that they may be able to spread the disease, before saying more it is necessary that the real virus of the disease should be demonstrated in them.

(5) FAECAL-BORNE THEORY OF BERIBERI.

It has been suggested that beriberi is spread through the faeces. If this were so we might expect that other "faecal-borne" diseases, when introduced, would be found to spread in coincidence with beriberi. Since both on Christmas Island and in Pudu gaol¹ cases of dysentery are introduced from time to time, and since this malady has shown no signs of spreading about amongst the inhabitants, it may be argued that this disease cannot have the same mode of spreading as beriberi.

The only disease, other than beriberi, which tended to spread on Christmas Island, apart from the stomatitis which has been mentioned, was a form of conjunctivitis or ophthalmia. This disease gave a good deal of trouble for a time and afterwards recrudesced again, if my memory serves me correctly.

Owing to the practice of manuring kitchen gardens by the Chinese with crude human sewage it might be thought that the vegetables might be the means of disseminating beriberi. But on Christmas Island though the coolies practically had no fresh vegetables at all, beriberi spread widely amongst them.

(6) THEORY OF EMANATIONS FROM SPECIFICALLY INFECTED SURROUNDINGS.

This theory has latterly been advocated by Sir Patrick Manson, and I regret that the observations which I have made are rather directly opposed to the acceptance of this theory of the mode of spread of beriberi.

A comparison may be made of the gaol at Taiping in Perak and that at Pudu in Selangor; though at one time seriously crippled with beriberi for some years there has been no trouble with the disease in recent times at the former, whilst, as has been pointed out above, the latter has been much ravaged. In general the Taiping climate appears to be rather more prevalently rainy and damp than Pudu. In general, there is not much to say about the buildings themselves, for although the cells are somewhat better at Taiping there is hardly enough difference to account for the circumstance satisfactorily. The differences which I

¹ The general cleanliness and tidy management of this gaol would be against the possibility of spread of a faecal-borne disease. In some gaols in India dysentery appears to be endemic.

was able to determine in the routines of these two gaols were (1) that clothes at the laundry were boiled or steamed, (2) all dried fish was steamed before being fried, (3) a tahl [$1\frac{1}{2}$ oz.] of dhal [kind of lentil] was given each day to each man in the rice, (4) blankets which were occasionally washed were used in place of sleeping mats. These methods were in vogue at Taiping but not at Pudu.

I did not witness the process of sweeping out the cells at Taiping, but at Pudu this was done dry, and much unpleasant dust was caused. Nor did I ascertain to what extent the prisoners were transferred from one to another cell comparatively in the two gaols.

The ventilation of the gaol cells at Pudu was certainly defective, but that this in itself was not the sole contributing cause of the beriberi is shown by the fact that the epidemic ceased before the widening of the ventilating slits was begun. During the next four months there were no further cases of beriberi, so it is presumable that the virus either ceased to exist or became latent independently of the ventilating arrangements. When looked at from the point of view of the possibility of some growth occurring in and about the cells, which could give rise to the supposed emanation, the difficulty was to locate where the possible nidus could be. Certainly there did not appear to be any opportunity for active growth of bacteria to take place; the white-washed walls, the simple wooden plank bed, the cemented floor and the ventilating slits were all far too dry for any ordinary bacterial growth to occur with vigour, that is if the conditions under which bacteria behave in the laboratory are of value as a criterion. The dust and "flue" which I collected from the beds, the floors and the ventilating slits gave a large and varied assortment of growths when diluted and plated. Curiously enough amongst the resulting colonies there were singularly few moulds. Many of the plates showed no moulds whatever. It may be considered certain that if such a source is the cause of beriberi, the organism must be different in character to the bacteria with which we are acquainted for a vigorous elaboration of toxic products to have occurred under these conditions. If now we contrast the ill-ventilated gaol cell with the tenements occupied by the sadly stricken coolies on Christmas Island, the site (see Plate X, Figs. 1 and 2) occupied by these houses is a good and airy one, the jungle has been cleared and is quite open to the cliff edge. The ground consists of fissured coral rock incompletely covered by soil, the natural drainage is good and no standing water was ever present near by. An idea of the houses themselves can best be obtained from the photograph Fig. 3. They are 38 in number, arranged

in a street (Fig. 2). The piles on which they are supported are arranged so as to put all floors on a level ; thus since there is a slight inclination of the ground, whilst there is just about head room under those at the near end, the floors of the further ones are 9 or more feet above the ground. The floor boards are supposed to be separated by crevices of a quarter of an inch, but as a matter of fact most of them are more widely separated ; the floor area measures roughly 35×16 feet. There is ample opportunity for ventilation through the floor.

The lowest strake of planks of the side walls is missing all round, so that there is a free air inlet about 9 inches wide between floor and wall.

The shutters are closed at night by the men, but still this does not impede the passage of air about the dwelling to an appreciable extent since the atap thatch of the simple roof is itself pervious to air. Yet more important, the whole of the gable ends are merely curtained by a screen of loose hanging ataps (this is well shown in the photograph Fig. 3). At a nocturnal visit to one of these houses it is difficult to speak of the freshness of the air owing to the usual presence of the heavy reek of opium ; still the conditions are such that there cannot be much stagnation and concentration of deadly miasmata. On the whole there was a tendency to dampness within ; this was due to some extent to defects in the atap thatch of the roof, and partly, for some days a week, during the wet monsoon, to non-evaporation of the sublimate lotion with which they were drenched week by week at my first visit, and fortnight by fortnight at the second visit.

Outside, the woodwork is tarred or pitched ; inside, the walls are whitewashed. To my mind unnecessarily, but at Government desire, the Company have concreted the areas under the houses ; but before this I did not detect any marked untidiness. Fig. 4 shows one end of the three hospital buildings which are situated close to the open ocean on a dry rocky site. The horizon on the ocean should show a little above the verandah rail, but it does not appear in the prints. The openness and airiness of the building may be best judged from the figure without entering into a lengthy description.

Now to my mind it is quite inconceivable that enough emanation could be concentrated in the air within these buildings to cause or protract disease. When it is remembered that the cooly does not spend more than 9 hours of the 24 within these buildings (except perhaps on Sundays, when many at any rate go out into the jungle after birds &c.), it becomes still more inconceivable that an emanation can be so potently

evolved that enough can be absorbed in about a third of the daily cycle to produce such grave illness; still more that its absorption should continue within the hospital buildings and lead to a fatal issue.

Thus by comparing the beriberi stricken inmates at the gaol¹ with the free air life of the phosphate cooly, we can but be driven to the conclusion that the matter of ventilation has no great or real determining influence.

VII. Conclusion and Summary.

Finally, I must express my hearty thanks to the many medical men and others who have given me aid and hospitality in my wanderings after the truth about beriberi. I must mention particularly Dr Giddy and Captain Vincent on Christmas Island, Drs Ellis, Leask and Murray Robertson in Singapore, Drs Travers, Gimlette, Watson, McClosky and Mr Galloway in Selangor, and Drs M. J. Wright and Connolly in Perak, and Messrs Mason, Ponsford, Sanderson, and Hembrey in Pahang, without whose aid and kindness I could have seen but little. I must also thank my friend Dr G. S. Buchanan, Secretary of the Royal Commission on Arsenical Poisoning, for forwarding me much information.

It would seem from my observations that certain of the current theories of the causation of beriberi will not account for the conditions which have been encountered by me. The *dietetic* or *physiological*, the *unsound food*, the *arsenical*, and the *emanation theories* all appear to be insufficiently in accordance with the attendant circumstances to have accounted for the spread of the disease.

It is suggested that certain articles of diet, by virtue especially of containing phosphorised and fatty matters, may tend to ward off the disease when given in sufficient amounts. So far as there was any semblance of a positive result in the observations it is suggestive that beriberi is communicated from person to person more or less directly or through fomites as an actual infection. This infection is not of the nature of a septicaemia (since the internal organs at death prove sterile), but to a surface condition about the upper air passages. From the observation of the throats of a number of patients it is surmised that the redness which is therein seen, especially in early cases, may be intimately connected with the disease. The appearances and disappearances of beriberi and the more or less seasonal waves of prevalence of the disease

¹ The day-time is mostly spent at work under open sheds.

are not unlike those which are seen in the case of the infective diseases which we meet with in this country. The proneness of the newcomer to suffer fits in also with such a view. The difficulty in tracing out the source of infection in a disease like diphtheria is often great, especially since we know that the causative organism may be carried by unsuspected refractory or immune persons or animals.

With considerations such as these it is held by the writer that there is no inherent impossibility in explaining many of the circumstances connected with the spread of beriberi.

Observed facts seem to show that beriberi should rather be considered in the light of a "gang" or "institutional" disease than as a "place" or "house" disease.

EXPLANATION OF PLATE X.

Fig. 1. Shows general view of the cooly lines on Christmas Island from "Loading Point."

The building on the extreme right is the store; next to it is the house occupied by the Chinese contractor's agents; a little below and to the left are two white roofs, these are open, covered areas of which the lower is the kitchen (next to which on the left is the house occupied by the cooks) and the one above is where the coolies mess. Still further to the left near the cliff edge is the bathing house, which owing to improved water supply can now I believe be used; annexed to it is now a room for drying clothes. The small erections used by way of w.-c.s. can be detected; the excreta are cast over the cliff into the ocean. On the point at the extreme left some of the hospital buildings can be perceived. The cove on the right has a sandy beach and is used by the coolies for sea-bathing.

Fig. 2. Shows the arrangement of the coolie houses as a street.

Fig. 3. Shows the construction of one of the houses in more detail; in front is a table used for gambling in the evenings till 9 p.m.

Fig. 4. Gives an idea of the site and construction of one of the hospital buildings. The shed below is the mortuary, where corpses are placed for not more than a few hours previous to their burial.

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Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4

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ON THE RESISTANCE OF THE MICROCOCCUS MELITENSIS TO MOIST HEAT.

SUGGESTED "STANDARD" METHODS IN THE DETERMINATION
OF THERMAL DEATH POINTS.

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THE effects produced upon bacteria by environmental conditions constitute factors which until recently have been studied with but too little regard to scientific accuracy, even by the bacteriologist investigating the conditions under which pathogenic bacteria occur in nature, and whilst probably leading a saprophytic existence.

Of such phenomena the "thermal death point" is one of the most important, and, at the same time, it is one which is often determined by methods that leave much to be desired. Occasionally it would almost appear that this biological constant has been fixed as the result of a process no more exhaustive than an effort of the imagination; whilst in the case of some even well-known micro-organisms this necessary information is entirely lacking from all descriptions of their life-history.

The *Micrococcus melitensis* falls under the last category, for after searching all the available literature, in quest of information on the subject of the thermal death point of this organism, all we have been able to gather is contained in the following sentence from "Mediterranean, Malta or Undulant Fever" by Surgeon-Captain Hughes, and published in 1897. The author there states (p. 42): "It (*i.e.* *M.*

melitensis grows best...at a temperature of about 37° C. At temperatures between 40° and 42° C. growth is suspended; above 42° C. artificial growths die."

We are quite in agreement with that portion of the quotation referring to the optimum temperature, but the latter part is so at variance with our experience (for *M. melitensis* is capable of fairly vigorous growth at 40° C. and also at 42° C., and only commences to show signs of lowered vitality when an attempt is made to cultivate the coccus at 44° C.) that we now wish to record the thermal death point as determined by us, and also to take this opportunity of describing the methods employed in our investigations—methods which we are inclined to believe are open to but few fallacies, and which we would suggest might be accepted, temporarily at any rate, as standards for the determination of the thermal death points of pathogenic bacteria in general.

These "methods" may conveniently be discussed under the headings of:

- (1) Time *v.* temperature.
- (2) Cultivation of organism for testing.
- (3) Source of heat.
- (4) Technique of the test.

1. *Time v. temperature.* Before proceeding further it is necessary to consider the precise meaning attaching to the term "thermal death point."

"Thermal death point" is, as we understand it, a term employed to express a definite and specific characteristic of some particular organism, but unfortunately the term is variously interpreted according to the importance attached to the time factor or to the thermal factor, respectively, by individual mycologists.

The majority of writers, it would appear, employ the term to express that temperature at which the vegetative form of the organism under observation is killed after an exposure of ten minutes; and this is the sense in which it was accepted by the Committee of American bacteriologists in their reports to the American Public Health Association in 1898. A few observers intend it to mean the lowest temperature that will cause instant death; others take a definite temperature, usually 60° C., and record the thermal death point as the time length of exposure; still others take neither a fixed time nor a fixed temperature, but record the lowest temperature that will certainly kill the organism after a comparatively lengthy exposure, varying with the taste of the

observer from thirty minutes to one hour. All observers are agreed, however, in employing moist heat in the test, for when dry organisms are tested their resistance to the lethal effect of heat will be found to vary according to the thoroughness of the desiccating process.

The relative importance of these two factors, time and temperature, appeared to us of the first moment, and after careful consideration we decided to adopt the first of the above-mentioned translations of the term, and to take as our primary standard a fixed time exposure of ten minutes, and to record as the death point that temperature which invariably caused the death of all the individual bacteria exposed to it for that period of time.

2. *Cultivation of organism for testing.* The next point for consideration is what cultivation of the organism under investigation should be employed. In order to obtain reliable and comparable results it is necessary to examine the organism at the period of maximum vegetative growth, that is to say, at a period when the cultivation contains the largest number of actively dividing virile elements and consequently on the one hand the smallest number of individuals of impaired vitality or already dead, and, on the other, of resistant resting forms. In order to fulfil this condition it is first necessary that the organism should be grown under optimum conditions as to (a) reaction of medium, (b) temperature, and (c) atmosphere; and for the majority of pathogenic, facultative anaerobic organisms, cultivations on solid media preferably upon the surface of nutrient agar (+ 10 Eyre's scale), grown aerobically at 37° C., will be found to yield the most suitable growths. When grown under optimum conditions the most suitable *age* at which to test cultivations in order to fulfil the requirements as to vitality, etc. is a point which can only be determined by actual experiment, but will generally be found to lie between 24 and 48 hours; sometimes, though but rarely, between 48 and 96 hours.

An even emulsion of such an optimum cultivation must be prepared in an indifferent diluting fluid such as sterile normal saline solution and transferred to sterile test-tubes, in which it can conveniently be exposed to moist heat of any desired temperature, as described in Section 4, p. 166.

In the case of the organism with which we are now concerned, *M. melitensis*, the optimum conditions were determined by a series of tests, which we do not propose to discuss here, to be as follows:

Tube smear cultivations upon the sloped surface of nutrient glycerine agar (5% glycerine) reaction +10, grown aerobically for a period of 48 hours at a temperature of 37° C.

3. *Source of heat.* The usual method of determining a thermal death point consists in subjecting the preparation of the micro-organism to the influence of water heated in an ordinary water-bath. The requisite temperature is maintained by constantly observing a sensitive thermometer which has its bulb immersed in the water, and controlling and adjusting the gas jet below the water-bath, either with or without the aid of some form of mercury thermo-regulator, in direct response to the fluctuations of the thermometer.

The response of the column of mercury in the thermo-regulator

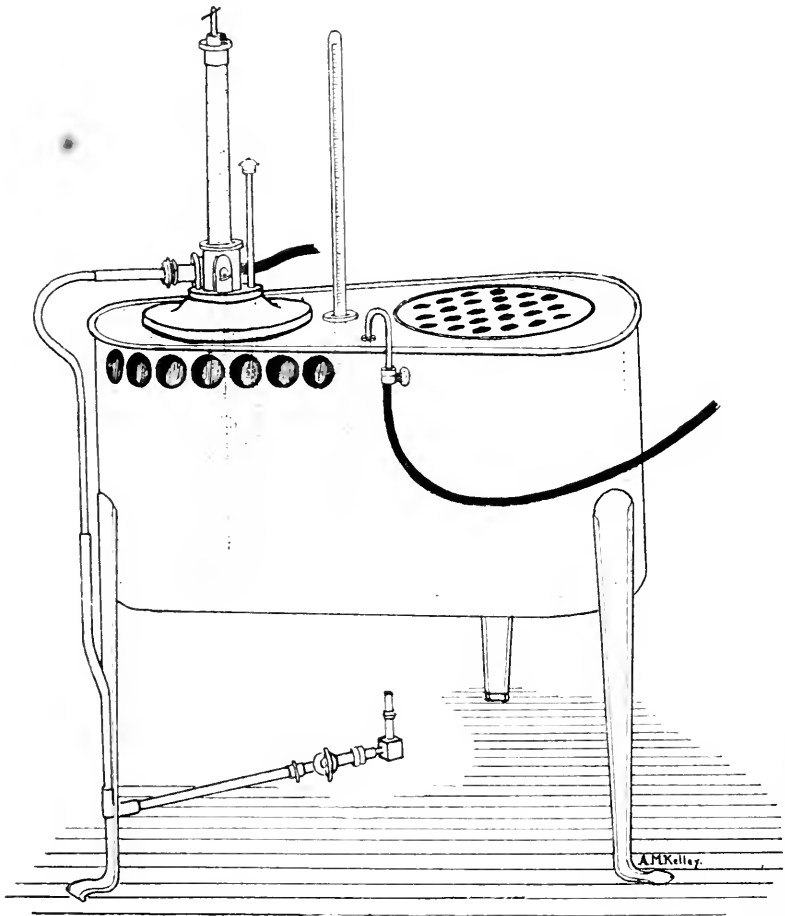


Fig. 1.

itself to variations in the temperature and pressure of the external air constitutes a serious objection to this method; and necessitates constant attention on the part of the observer who employs it. This objection is painfully obvious when a series of observations, say at intervals of two degrees from 50°C. to 65°C. , have to be carried out in one bath. In such cases the trouble and annoyance, to say nothing of the difficulties of adjustment, and the consequent loss of time, are excessive: so much so in fact that some twelve months ago one of the present writers considered the possibility of utilising one of Hearson's Excelsior gas valves combined with a thermostatic capsule for the purpose of regulating the temperature of the water in the bath—previous experience with biological incubators having conclusively proved the value of this form of thermo-regulator. As the result of many consultations with Mr Charles Hearson and a considerable amount of experimental work on the part of that gentleman, a water-bath has been evolved which is so satisfactory and reliable for determining thermal death points that a full description of this piece of apparatus may suitably be given here. In shape and general appearance this water-bath is very similar to the well-known vacuum embedding apparatus made by the same firm. As will be seen from the accompanying figure (Fig. 1), the apparatus consists practically of a large enclosed water-bath, roughly oval in shape, but decidedly broader at one end, made of copper and enclosed in a loose casing of sheet-iron lined with asbestos, and mounted on three legs. The narrower end is entirely occupied by the automatic gas valve, which modifies the gas supply to the flame which burns below the bath and by means of which the water is heated.

The broad end of the water-bath, which is available for experimental work, equals in its capacity a cylindrical vessel 20 centimetres in diameter by 12 centimetres deep. The mouth is closed by a circular copper disc perforated by 31 holes, each 2 centimetres in diameter, in which the tubes containing the material under examination are suspended.

The action of this gas regulator as adapted for thermal death point work will readily be appreciated by a reference to Fig. 2. The capsule *A* which is immersed in the water, is a flat metal box containing a few drops of fluid boiling at a certain definite temperature, viz. 45°C. This is suspended in a brass frame, which is attached in its turn to the gas valve above by means of a tube *B*. The gas valve is formed by two flattened circular brass castings *D* and *E* accurately adjusted and fastened together firmly by means of screws. The lower casting is

box (*C*) for the support of the thermostatic capsule. A loose metal rod contained in the tube (*B*), its lower end resting in a cup-shaped hollow on the upper wall of the capsule, serves to transmit the expansion of the capsule to the gas valve.

Through the axis of the upper casting a hole about half-an-inch in diameter is bored and screwed at its upper part for the reception of the brass tube (*G*) carrying a long spiral spring. The lumen of this tube, continuous with the central cavity of the casting, forms to all intents and purposes a gas reservoir. On the lower surface of the casting is a deep groove (*H*) concentric with the central chamber. The casting is provided with a brass tube (*I*) establishing communication between the lumen of the channel and the exterior, and serving for the entry of gas. The groove and the lower end of the central orifice are closed by means of an obturator membrane (*K*) which is held firmly in position between the opposing surfaces of the casting. A small-bore channel is also drilled from the outside of the casting into the central chamber, communicating by means of a right-angled branch with the circular groove and tapped to receive a screw (*L*). This forms a needle valve by-pass. The rush of gas through the entry tube fills the groove, and by its pressure depresses the obturator, and so passes on into the central chamber, and from there out by way of the tube (*M*) to the gas jet below the bath.

The regulation by means of the capsule comes into play as soon as the water in the bath is sufficiently hot to cause the fluid in the capsule (*A*) to boil, when the walls of the metal box expand and drive the metal rod (*N*), resting on capsule *A*, against a brass disc on the under side of the obturator. When this upward pressure on the obturator is sufficient to overcome the resistance of the incoming gas the way is stopped, and only such gas as filters up through the needle valve by-pass can reach the central chamber and so get to the gas jet, and prevent total extinction of the flame. The tube (*B*) is slotted to permit connection of the lever rod (*N*) with a brass arm (*O*) which is curved upwards and passes through the expanded plate of the gas valve. As only a small amount of "play" is permitted to this arm, an upward pull on its knobbed extremity (*Q*) shows at once whether the valve is working satisfactorily or not, and also, by the amount of movement necessary to cut off the main supply of gas to the jet below the bath, how near the temperature of the water in the bath is to the boiling point of the thermostatic capsule.

To raise the pressure of the fluid in the capsule and so the boiling

point of the contained fluid it is necessary to compress the spiral spring contained in the upper tube (*G*) between the sliding plunger (*P*) and the metal plate on the upper surface of the obturator. This is effected by turning the rod (*R*) which passes through the head of a long screw (*S*) in a direction contrary to that of the hands of a clock, *i.e.* contra-clockwise, and so driving the plunger downwards. The pressure is then transmitted through the lever rod (*N*) to the walls of the capsule. Thanks to the length of the spiral spring, sufficient pressure can be exerted on the walls of the capsule to raise the boiling point of the contained fluid some 20° C. As a result of the increased pressure of the fluid in the capsule and the consequent rise in its boiling point, a higher temperature is necessary before the water in the bath is hot enough to cause the capsule to act and cut off the main gas supply. By turning the metal arm "clockwise" the spiral spring is released and the pressure on the capsule being relieved, the boiling point of the contained fluid again falls, and the water in the bath can be regulated for a lower temperature. The method of setting the valve when commencing work may be briefly described in the following steps:—

1. Allow the spiral spring to expand to its fullest extent by twisting the metal arm (*R*) "clockwise" until arrested by the stop.
2. Disconnect the valve by unscrewing the union connecting the gas exit pipe (*M*) with the tube leading to the gas jet beneath the bath, and remove the regulator *en bloc*.
3. Screw the needle valve (*L*) quite in, so that there shall be no passage in that direction. Blow through the gas supply tube (*I*), and while so doing, pull up the knobbed arm (*O*) and notice a sudden stoppage of the gas way.
4. Release the knobbed arm, and, while still blowing, take hold of the capsule box (*C*), and turn in the direction to screw the tube into the lower casting *E*. Soon there will come a moment easily distinguishable when the passage of air will be cut off. Having found this point, give the whole capsule box and tube half a turn backwards. The whole object of this operation is to set the end of the lever rod (*N*) a known distance from the valve obturator, for when this adjustment is properly made it is obvious that an expansion of the capsule, equal to half the distance between one thread and another on the tube (*B*), will close the valve.
5. Pour water, not hotter than 40° C., into the water-bath until it reaches to within three or four centimetres of the top.
6. When satisfied that the adjustment has been properly made, lower the capsule box into the water-bath and connect the tube (*I*), which up to this time has only been blown through, with the gas service pipe.
7. Connect the gas exit pipe (*M*) with the metal tube which goes to the burner under the bath, by means of the screw union.
8. Turn on the gas and light the luminous jet beneath the bath, reduce the flame to the size of the bowl of a dessert-spoon.

9. Withdraw the screw valve (*L*) two or three turns.
10. Pull up the knobbed wire (*Q*), and, whilst thus cutting off the main supply at the valve, turn the screw (*L*) in the direction to reduce the flame until it is not larger than a pea.
11. Slowly work the knobbed wire (*Q*) up and down, and notice the variation in the size of the flame, representing in one case the full supply through the main valve, and in the other case the small flame through the needle valve by-pass.
12. Release the knobbed wire, and if the temperature of the water in the bath is still below the acting temperature of the capsule the large flame will appear, and will remain until the water is hot enough to make the capsule act.
13. When this point is reached the capsule will expand and push up the lever rod *N* and the main supply will be cut off, just as when the knobbed wire is pulled upon; whilst a thermometer inserted through a hole in the cover of the bath (see Fig. 1), with its bulb immersed in the water of the bath, will record the temperature at which the capsule is acting.

Once the bath is regulated the desired temperature will be maintained with less than half a degree variation above or below, for months at a time if need be, without further attention than replacing the water lost by evaporation. Variations in the pressure of the external air and variations in the temperature, unless excessive, appear to have no influence on the efficiency of the regulator.

A point of some importance, especially in connection with thermal death point determinations, is the fact that in an ordinary water-bath the water tends to collect in layers or strata having slightly different temperatures. To such an extent is this true that the water at the bottom of a deep bath may differ by one degree from that at the surface. Fallacies due to this cause may be eliminated by keeping the water in the bath in constant movement. In the water-bath we have used for our experiments we have provided a second hole in the cover of the bath at some little distance from that occupied by the thermometer. Through this is passed a piece of glass tubing drawn out to a fairly fine point (see Fig. 1). The other end of the glass tube is connected up to the nozzle of a "water blower" by a short length of india-rubber pressure tubing and a fine jet of air driven into the water of the bath the whole time an experiment is in progress. By this means the water is effectually prevented from stratifying and more consistent and reliable results are obtained.

[*Note.* By replacing the perforated copper disc which closes the mouth of the water-bath by the cylindrical copper vessel usually supplied with Hearson's vacuum embedding bath, and loosely covering the plate-glass lid of this vessel with a layer of felt, a small incubator can be improvised for determining the "optimum" temperature for cultivation of mesophilic and thermophilic bacteria. In this work

however it may be necessary to experiment at temperatures as low as 25° C. or as high as 80° C. or even higher. One thermostatic capsule is insufficient for this purpose, but as the capsules are readily interchangeable it is quite simple to obtain a set of three, the lowest one ranging from 30° C. to 50° C., the next from 45° C. to 60° C., and the third from 55° C. to 75° C., while a short thermometer graduated from 20° C. to 100° C. can be fixed, scale upwards, in contact with the under surface of the glass lid of the vessel by means of two or three strips of sticking-plaster (a method which allows the column of mercury in the thermometer to be readily observed by simply raising the felt covering), and completes the incubator.]

4. *Technique of the test. Preparation of the emulsion.* For the purpose of testing the thermal death point a homogeneous emulsion is first prepared from what we may term the "optimum" culture of the organism under study, and in doing this, care must be taken to make every series of experiments comparable by using definite quantities of culture and of diluting fluid, and controlling this method by enumerating the cocci present per cubic centimetre of the emulsion. For this purpose a platinum loop must be especially and carefully made, calibrated to contain about two milligrammes of the growth from an optimum culture. That the exact weight of cultivation is removed by the loop, is of less consequence than that the loop should be filled in the same manner each time it is used. Simply a film gathered up in the loop is insufficient,—the loop should be so filled with the growth that a biconvex mass is supported in the area enclosed by the loop. To prepare the emulsion a few—two or three—cubic centimetres of sterile salt solution (0.6 per cent. sodium chloride in distilled water) should be pipetted into a sterile glass capsule having a capacity of about 15 c.c., and the inner surface of one side of the capsule moistened with the solution. The loop charged with cultivation is now passed into the capsule and the germ growth rubbed off on the side of the glass and worked up with the smallest possible quantity of salt solution into a thin paste. By gentle movements of the loop this paste is incorporated with the bulk of the salt solution and thoroughly mixed. This process is repeated five times. Sterile salt solution is now added to bring the bulk of the emulsion up to 10 c.c. The resulting emulsion is thus practically equivalent to a suspension of 1 milligramme of cultivation per cubic centimetre. The emulsion is next filtered through a sterile paper-filter to remove any clumps that may be present in it, and 3 c.c. of the uniformly turbid filtrate pipetted into each of three plugged and sterilised test-tubes.

Bulk of emulsion. The bulk of the emulsion to be tested is of some importance too—to make a very much larger quantity of emulsion

takes a considerable time, a smaller quantity tends to evaporate whilst even at comparatively low temperatures, leaving the germ contents in a dry film on the inner surface of the test-tube. A quantity of 3 c.c. will make a column of fluid, in such test-tubes as we use, of about 1.5 cm. Such a quantity undergoes no appreciable diminution in bulk as the result of evaporation after an exposure of 10 minutes even to a temperature of 65° C.

Germ contents of the emulsion. The cubic centimetre of emulsion remaining in the glass capsule is now plated in order to determine the number of organisms present in the emulsion. This process is carried out as follows:—

Two glass capsules, each of 15 c.c. capacity, are taken, and numbered 2 and 3, respectively; and into each is pipetted 9.9 c.c. of sterile salt solution. To the contents of capsule No. 2 is added 0.1 c.c. of the emulsion and thoroughly mixed. Next 0.1 c.c. of this mixture is added to the salt solution in capsule No. 3 and also thoroughly mixed. The contents of capsules 2 and 3 now represent hundred-fold and ten thousand-fold dilutions respectively of the original emulsion, and, if 0.1 c.c. of each is plated in nutrient agar, or better still 0.2 c.c., 0.3 c.c. and 0.5 c.c., as then the counts of the various plates can be made to control each other, incubated at 37° C. for 48 hours, and the resulting colonies enumerated, a fairly accurate estimate can be made of the number of organisms originally present per cubic centimetre of the emulsion, or per milligramme of cultivation.

Test-tubes. The size of the test-tubes used to contain the emulsion which is to be subjected to the action of the moist heat is, in our opinion, of less importance than the thickness of its walls. Those we employ are of the variety known as stout, and measure 18 centimetres in length and have a diameter of 1.5 centimetres, while the thickness of the walls is about 1 mm. To employ test-tubes of thinner glass than this is to run the risk of transmitting the heat too quickly, thereby leading to the evaporation of the small quantities of fluid which frequently adhere to the glass just about the upper limit of the bulk of the emulsion. When this happens the organisms previously suspended in that fluid become more resistant to heat as the result of their desiccation, with the consequence that fallacious results are obtained. The contents of thick walled test-tubes on the other hand are raised in temperature in a more uniform manner, and any of the emulsion accidentally splashed on the sides of the tube has an opportunity of

trickling down and rejoining the main body of emulsion before any marked elevation in temperature has taken place.

A preliminary test is now carried out to determine the thermal death point to within 5°C . by exposing a separate tube of the emulsion to each of several temperatures varying to that extent, say— 45°C ., 50°C ., and 55°C . The water-bath is started as described in Section 2, with the spring controlling the pressure in its thermostatic capsule unwound and quite slack, and the water blower turned on to admit the current of air into the water in the bath. The perforated cover is placed on the bath, and each of the holes is closed by means of a vulcanite ball or a glass solitaire "marble" to prevent evaporation. As soon as the temperature of the water in the bath has reached 45°C ., as indicated by the thermometer suspended through the orifice in the bath lid, the capsule acts and cuts off the main supply of gas to a greater or lesser extent, depending on the temperature of the external air, the temperature frequently being maintained by the jet supplied by the needle valve alone. The perforated bath-cover is now taken off and given to an assistant to hold.

Experiment 1. Remove one of the balls closing a hole in the perforated bath-cover and replace it by a test-tube containing the emulsion. The method of slinging the tubes in this rack is extremely simple. A thick rubber ring is placed round the tube to form a shoulder, and the tube lowered into position in its particular hole in the disc. A second rubber ring is then slipped on to it from below and pushed up to the under surface of the disc. The tube is now firmly fixed so that it can neither float upwards when immersed in the water of the bath nor slip down through the hole in the disc and sink into the bath. Any holes in the disc not occupied by test-tubes are occluded by means of vulcanite balls, or even common clay or glass marbles, in order to prevent loss of temperature and unnecessary loss of water by evaporation. Now measure and if necessary adjust the depth of water in the bath, and so arrange the test-tube that the upper limit of the emulsion in the tube is submerged to the extent of about 4 centimetres, while the bottom of the tube is a like distance above the bottom of the water-bath. At the same time arrange a second similar tube, containing 5 cubic centimetres of sterile salt solution and provided with a cotton-wool plug through which a thermometer is passed so that its bulb is immersed in the salt solution, in the same way in another hole in the immediate vicinity of the tube containing the emulsion. The temperature of the water-bath being now 45°C ., replace the cover and observe the thermometer resting in the sterile salt solution. As soon as the temperature of this salt solution has risen to 45°C . and the thermometer immersed therein corresponds with that recording the temperature of the water in the bath, note the time, and 10 minutes later remove both test-tubes from the bath and rapidly cool down to the room temperature by holding their lower halves under a stream of running cold water.

As soon as the emulsion has cooled down to 20° C., remove 1 cubic centimetre from the test-tube by means of a graduated sterile pipette, and add 0.2 c.c., 0.3 c.c., and 0.5 c.c. respectively to each of three tubes of melted agar which has been cooled to 42° C., and pour three plates.

Now pipette 10 c.c. sterile broth into the tube containing the remaining 2 cubic centimetres of emulsion; and incubate both broth tube and the set of agar plates aerobically at 37° C. for 48 hours. At the end of this time count the colonies appearing on the agar plates, when the total of the three plates gives the number per cubic centimetre of cocci which have resisted the effect of a 10 minutes' exposure to 45° C., while a comparison with the figures obtained as a result of plating the original unheated emulsion will show how many cocci, if any, have succumbed. (If the organism under examination is one which does not give typical naked eye colonies, or colonies that can be easily recognised with the aid of the low power of the microscope, many subcultures should be made from the colonies appearing after incubation, and the organism identified.) The broth cultivation must also be observed, and the growth, if any, observed microscopically to determine its purity and finally subcultures made in order to identify the organism. If no growth has appeared either in the broth cultivation or on the agar plates the period of observation is extended to at least 7, or better 14, days; as individual organisms, though not killed by exposure to any given temperature, may be so weakened by that exposure that some time elapses before they are sufficiently recovered to reproduce themselves in numbers to give rise to any macroscopical growth.

Experiment 2. Having completed the experiment at 45° C., the bath is reset for a temperature of 50° C. This is effected by screwing the metal arm (Fig. 1 and 2) contra-clockwise until the gas flame under the bath suddenly rises to its full height, indicating that the boiling point of the fluid in the thermostatic capsule is now higher than the temperature of the water in the bath. In a few minutes the water will be heated sufficiently to again cause the capsule to act and cut off the main gas supply. The temperature recorded by the thermometer immersed in the water of the bath is observed and will probably be found to be 45.5° C. The spring must be compressed again until the gas flame rises, and this process repeated until the thermometer records a temperature of 50° C. The adjustment is now complete¹, and the bath is ready for the next experiment, which is conducted exactly as described under Experiment 1, applying the higher temperature to the emulsion contained in the second of the test-tubes.

Experiment 3. The water-bath is now adjusted to a temperature of 55° C. and the third experiment carried out precisely as in the two former instances.

In the event of a growth taking place on either agar plates or broth tube after exposure of the emulsion for 10 minutes to a temperature of 55° C. a further set of experiments is performed at higher temperatures, 60° C., 65° C., and 70° C., and the two temperatures between which growth ceases are now fixed, and finally a set of experiments consisting of exposure of the emulsion to temperatures varying only

¹ The adjustment of the water-bath at a higher temperature can be hastened by placing a lighted Bunsen burner beneath the broad end of the bath, in the intervals between the successive compressions of the spiral spring.

1° C., from the highest at which growth was observed to the lowest which killed all the organisms contained in the emulsion. Further exactitude can be obtained if desired by again testing emulsions of the organisms at temperatures varying only 0·5° C., and the results obtained must be controlled at least once, preferably two or three times, before the thermal death point of the organism is finally recorded as situated between a certain degree Centigrade and the half degree immediately above.

The thermal death point of Micrococcus melitensis. Having described our methods in some detail—methods which have been elaborated by one of the writers as the result of many experiments and observations spread out over a period of about two years—it now only remains to record the observations on the resistance of the *Micrococcus melitensis* to moist heat, from which we have deduced the thermal death point of the organism. Our results can be shown most easily in tabular form, but before giving these tables, we may be allowed to refer to each of the various “strains,” five in number, we utilised for the experiments.

Strain No. 1 was derived from a cultivation of the *Micrococcus melitensis* obtained early in 1900 from Dr Zammit through the kindness of Staff-Surgeon Stonehouse, R.N., with the real source of which we are unacquainted, beyond the fact that it was isolated from the human spleen post-mortem. This culture has been under cultivation in the laboratory for at least four years.

Strain No. 2 was derived from the spleen at the post-mortem of a fatal case of Malta fever, and was given to one of us (J. W. H. E.) by Dr Zammit, of the Public Health Service, Malta, towards the end of 1901, and has been under cultivation for at least two years.

Strain No. 3 was also obtained through the kind courtesy of Dr Zammit, who sent the culture obtained post-mortem from the spleen of a fatal case of Malta fever to one of the writers early in 1902 by Dr Micallef, and has been under cultivation in the laboratory for more than eighteen months.

Strain No. 4 was isolated post-mortem by one of us (F. J. A. D.) from the spleen of a fatal case of Malta fever in January 1903, and has therefore been under cultivation in the laboratory 13 months. That the specific pathogenic properties of this strain have been fully maintained is sufficiently evidenced by the fact that accidental inoculation in July last with a minute quantity of an emulsion prepared from a cultivation of this coccus sufficed to set up a short though typical attack of Malta fever in one of the writers (J. W. H. E.), in which the serum reaction on the sixth day of the disease was immediately obtained in dilutions of at least 1 in 200.

Strain No. 5 was isolated (Sept. 1903) by one of us (J. W. H. E.) from the pus of a diaphragmatic abscess (in which the *Micrococcus melitensis* existed in pure cultivation) occurring in a man invalided home from Malta as suffering from the fever, and whose serum gave an immediate reaction of 1 in 200. A second pure cultivation of this organism was obtained three days later from the spleen at the post-mortem. This strain had only been under cultivation for two months, and in

point of fact the actual cultivation employed in the preparation of the emulsion was the sixth generation only from the original culture from the spleen.

Each of the above strains was quite typical of *M. melitensis* in morphology, staining reaction, and cultural characters. All were pathogenic (in fairly large doses) for rabbits and for guinea-pigs whether inoculated intravenously or intracerebrally.

In the following tables the result of the test made with each strain is shown, and the early gradual decrease and the later rapid decrease in the cocci remaining alive after the 10 minutes' heating can be compared with the actual number of cocci present per cubic centimetre of the unheated emulsion.

The figures given are those of actual experiments which approximate most closely to an average of the eight complete sets of observations that we have made. These numerous control observations were made in order to obtain results as accurate and as exact as such biological experiments can be made to yield.

The result of these observations, therefore, leads us to fix the thermal death point of *M. melitensis* at 57.5° C.

TABLE I.

Strain of organism employed	No. of bacteria present per c.c. of emulsion	45° C.		50° C.		55° C.	
		Broth	Agar: surviving cocci per c.c.	Broth	Agar: surviving cocci per c.c.	Broth	Agar: surviving cocci per c.c.
M. melitensis 1	2,750,000	+	2,000,000	+	450,000	+	3,000
M. melitensis 2	4,000,000	+	3,800,000	+	621,760	+	5,600
M. melitensis 3	1,700,000	+	2,000,000	+	110,500	+	1,320
M. melitensis 4	5,420,000	+	5,200,000	+	497,600	+	10,640
M. melitensis 5	7,050,000	+	6,900,000	+	1,000,000	+	8,000

TABLE II.

Strain of organism employed	No. of bacteria present per c.c. of emulsion	60° C.		65° C.		70° C.	
		Broth	Agar	Broth	Agar	Broth	Agar
M. melitensis 1	2,750,000	—	—	—	—	—	—
M. melitensis 2	4,000,000	—	—	—	—	—	—
M. melitensis 3	1,700,000	—	—	—	—	—	—
M. melitensis 4	5,420,000	—	—	—	—	—	—
M. melitensis 5	7,050,000	—	—	—	—	—	—

TABLE III.

Strains of organism employed	No. of bacteria present per c.c. of emulsion	56° C.		57° C.			58° C.		59° C.	
		Broth	Agar : surviving cocci per c.c.	Broth		Agar : surviving cocci per c.c.	Broth	Agar	Broth	Agar
				48 hrs	72 hrs					
M. melitensis 1	3,200,000	+	120	—	+	6	—	—	—	—
M. melitensis 2	4,100,000	+	122	—	+	3	—	—	—	—
M. melitensis 3	7,700,000	+	450	+	—	38	—	—	—	—
M. melitensis 4	6,300,000	+	300	+	—	10	—	—	—	—
M. melitensis 5	5,300,000	+	318	—	+	0	—	—	—	—

TABLE IV.

Strains of organism employed	No. of bacteria present per c.c. of emulsion	56·5° C.		57° C.		57·5° C.	
		Broth	Agar : surviving cocci per c.c.	Broth	Agar : surviving cocci per c.c.	Broth	Agar
M. melitensis 1	3,200,000	+	24	+	0	—	—
M. melitensis 2	4,100,000	+	70	+	2	—	—
M. melitensis 3	7,700,000	+	200	+	12	—	—
M. melitensis 4	6,300,000	+	130	+	2	—	—
M. melitensis 5	5,300,000	+	156	+	3	—	—

+ = growth

— = no growth

SUGGESTED STANDARDS.

1. Length of "*time exposure*" to be 10 minutes.
2. Emulsion to be prepared from "*optimum cultivation*."
3. The *vehicle* in which the culture is suspended to be sterile salt solution or sterile distilled water.
4. *Strength* of emulsion to correspond to about 1 milligramme of culture per cubic centimetre.
5. *Bulk* of emulsion to be not less than 3 c.c.
6. Emulsion to be contained in test-tube of 1·5 cm. diameter, with walls 1 mm. thick.
7. Emulsion to be exposed to moist heats in a water-bath regulated by a delicate and accurate thermo regulator—such as Hearson's thermo-static capsule.
8. Thermal death point to be first roughly determined to within 5° C.
9. Thermal death point finally fixed to within 1° C., as that temperature which causes the death of *all* the micro-organisms exposed to it, within the 10 minutes.

This set of experiments must be repeated at least once.

10. Broth cultivations and agar plates both to be used in determining the death of the bacteria: and the period of observation of these cultures to be extended, when necessary, to seven or fourteen days.

THE BACTERIOLOGICAL EXAMINATION OF OYSTERS AND ESTUARIAL WATERS.

By A. C. HOUSTON, M.B., D.Sc.

THE following paper embodies the main facts obtained during a prolonged investigation as regards the pollution of Tidal Waters and of Shell Fish, undertaken on behalf of the Royal Commission on Sewage Disposal¹.

The subject will be considered under the following five headings:—

- I. Bacteriological Examination of the River Thames and the Thames Estuary p. 173
- II. Passage of *B. pyocyaneus* through a continuous filter bed and through a septic tank and contact beds p. 176
- III. Bacteriological Examination of unpolluted sea-water p. 177
- IV. Bacteriological Examination of the Helford and Penryn Water and Oysters p. 180
- V. (Appendices A to L.) Results of a number of separate bacteriological observations bearing on the general question of the pollution of estuarial waters and shell-fish p. 188

I. *The Bacteriological Examination of the River Thames and the Thames Estuary.*

This portion of the inquiry involved the bacteriological examination of:—

(1) The water of the River Thames at Sunbury and Hampton above the intakes of some of the London Water Companies. (2) Filtered water as supplied to the consumers by the Southwark and Vauxhall and by the East London Water Works Companies. (3) The sewage effluents discharged into the Thames at Barking and Crossness. (4) The River Thames at (a) Barking, and at (b) Crossness; at (c) Purfleet; at (d) Grays; at (e) Mucking; and (f) at the Chapman

¹ Royal Commission on Sewage Disposal; Fourth Report; Vol. III; Reports by Dr Houston on Bacteriological Investigations.

Lighthouse. Also, (5) the bacteriological testing of (a) the Barking and Crossness chemically-precipitated sludge, and (b) the water in the Barrow Deep into which this sludge is discharged.

Briefly, the results may be summarised as follows:—

B. coli (or coli-like microbes) per c.c.

River Thames at	Crossness	}	About 100 to 1000 per c.c.
	Purfleet		
	Barking		
	Grays.		10 to 1000, but more usually 100 per c.c.
	Hampton	}	Usually 10 to 100, but more often 10 than 100 per c.c.
	Sunbury		
	Mucking.		1 to 100; more often 10 than 1; but very seldom 100 per c.c.
	Chapman	}	Ranged usually from 1 in 10 c.c. to 1 in 1 c.c.
	Barrow Deep		

Spores of *B. enteritidis sporogenes* per c.c.

River Thames at	Crossness	}	Usually 1 to 10, but more often 10 than 1.
	Purfleet		
	Barking		
	Grays		
	Mucking.		1 to 10, but more often 1 than 10.
	Barrow Deep	}	1 per 10 c.c. to 1 per 1 c.c., but rather more often the latter.
	Sunbury	}	1 per 10 c.c. to 1 per 1 c.c., but rather more often 1 per 10 c.c.
	Hampton		
	Chapman.		Positive result with 1 c.c. only in about every fourth sample.

Crossness, Purfleet, Grays, Mucking and Chapman are about 2, 7, 12, 22, and 27 miles respectively below Barking. Sunbury and Hampton are above the intakes of some of the London Water Companies. The Barrow Deep begins about 13 miles seawards of the Nore. During 1902 the combined volume of effluent from the Barking and Crossness works amounted to a daily average of 232 million gallons. About 50,000 tons of sludge are deposited weekly in the Barrow Deep.

The chemical effluents at Barking (Northern outfall) and Crossness (Southern outfall) contained usually 100,000 *B. coli* (or coli-like microbes) and 100 to 1000 spores of *B. enteritidis sporogenes* respectively per c.c.

The chemically-precipitated sludge at Barking (Northern outfall) and Crossness (Southern outfall) contained usually 1,000,000 to

10,000,000,000 *B. coli* (or coli-like microbes) and 10,000 to 100,000 spores of *B. enteritidis sporogenes* respectively per c.c.

The filtered water as supplied to the consumers by the Southwark and Vauxhall and by the East London Water Works Companies usually contained *B. coli* (or coli-like microbes) in 100, in 10, and sometimes even in 1 c.c.

Conclusions.

The water of the River Thames at Sunbury and Hampton above the intakes of some of the London Water Companies is most unsatisfactory from the bacteriological point of view.

The Barking and Crossness "chemically-produced" effluents resemble in their biological composition raw sewage.

The bacteriological condition of the River Thames at Barking, Crossness and Purfleet is very unsatisfactory.

At Grays, the water showed some slight evidence of improvement.

At Mucking, the Thames water showed definite signs of improvement.

At the Chapman Lighthouse the Thames water was so far improved relatively, as seemingly to vie in biological purity (*qua* *B. coli*) with some of the samples of filtered London water.

The alleged *gross* pollution of the Essex and Kent foreshores Eastwards of a line connecting the Chapman Lighthouse with Stoke, as a result of the discharge of the Barking and Crossness effluents into the Thames, would thus appear from these data to be without sufficient warrant.

The water obtained from the Barrow Deep was found to be, under the circumstances, remarkably satisfactory. The alleged *serious* pollution of the Thames Estuary, as a result of the deposit of sludge by the London County Council in the Barrow Deep, is not supported by the results of the bacteriological analyses.

As regards the filtered London (*main*) water (Southwark and Vauxhall and East London Water Companies) it is undesirable to speak definitely. Many of the coli-like microbes were atypical in character, nevertheless, the results were unsatisfactory from the bacteriological point of view.

Speaking in general terms the results indicate :—(1) That the water of a tidal river grossly polluted in its lower estuarial reaches may after a flow of 25 miles become so far purified by sedimentation, dilution, and

the operation presumably of bactericidal agencies, as to become seemingly as little objectionable, or in some respects less objectionable, bacteriologically than certain of our public water supplies. (2) That the deposition in the sea of chemically-precipitated sludge in enormous quantities, if carried out under proper conditions, need not result necessarily in the production of nuisance or serious pollution of the surrounding water, and that such deposition may be thought of as an economical and seemingly not unsatisfactory means of disposing of this material. (3) The danger of hastily condemning waters and other materials without a wider knowledge of comparative bacteriology and of the correlation of bacteriology and epidemiology than is at present available.

II. *The Inoculation of Sewage with B. pyocyaneus and the subsequent isolation of this microbe from (1 a) the effluent from a continuous filter ; (2 a) septic tank liquor ; and (2 b) the effluent from contact beds.*

In the case of the continuous bed *B. pyocyaneus* appeared in the effluent within *less than ten minutes* from the start of the experiment and was present (at first invariably, later at irregular and increasingly rare intervals) for some considerable time afterwards (*at least 5 hours* in the first experiment ; ten days in the second experiment).

In the case of the septic tank and contact beds *B. pyocyaneus* appeared in the septic tank liquor *within 2½ hours* from the start of the experiment, and in the contact bed effluents at the earliest possible time, *i.e.*, the first emptying of the bed after the passage of *B. pyocyaneus* through the septic tank and into the beds. In the first experiment *B. pyocyaneus* was still present in the septic tank liquor 24 hours from the start of the experiments and in the contact bed effluent up to the third day. In the second experiment, which was carried over a longer period, *B. pyocyaneus* was isolated from the septic tank liquor and from a contact bed effluent on the ninth day.

The results indicate the inadvisability of relying on septic tanks, contact beds, and continuous filters to remove altogether the element of potential danger to health associated with the discharge of effluents from these processes of sewage treatment either into *drinking-water* streams or into estuarial waters in the neighbourhood of shell-fish layings. This statement in no way implies that these processes of

sewage treatment are not capable of yielding satisfactory effluents from the chemical and practical point of view and in relation to *non-drinking-water* streams.

III. *The Bacteriological Examination of Samples of Unpolluted Sea-water.*

Thirty-four samples of deep-sea water were collected on the North-west coast of Scotland within sight of land though remote from all possibility of contamination at separate spots along a line more than 100 miles in length.

The results showed conclusively that *B. coli* (or coli-like microbes) may be absent from as large a volume as 100 c.c. of unpolluted sea-water. The *B. enteritidis sporogenes* test likewise yielded negative results when using 10 c.c. of the water for cultural purposes.

Laboratory experiments were also carried out to ascertain the vitality of *B. coli* in *pure* water kept at a definite temperature (20° C.). Four experiments were made with sea-water and six with tap-water. The results showed that *B. coli* added to sea-water and tap-water in large amount was no longer capable of demonstration in 1 c.c. of the sample after a maximum of nine days and a minimum of three days. A negative result with 1 c.c. does not mean necessarily the absolute death of *B. coli*, but it certainly indicates the relative disappearance of this microbe from the sample. The persistence of *B. coli* in polluted water and in mud is a separate question.

At the present time the attempt to lay down absolute standards is not justifiable, but the division of waters into classes, with tentative standards for comparative purposes, is perhaps permissible. In this connection the following tabular statement may be of interest to fellow-workers (see Table I. p. 178).

Conclusions.

1. *B. coli* and the spores of *B. enteritidis sporogenes* are absent from 100 c.c. and 10 c.c. respectively of multiple samples of unpolluted sea-water.
2. *B. coli* and *B. enteritidis sporogenes* are commonly present in 1/100,000 c.c. and 1/100 to 1/1,000 c.c. respectively of sewage.
3. Taking in account both these extremes, estuarial waters,

TABLE I.

CLASS*	Standard based on numerical abundance of <i>B. coli</i> (or non-liquefying, gas-forming coli-like microbes)	Numerical standard confirmed or modified according to response of the coli-like microbes in <i>pour-plate</i> to certain well-known biological tests	Provisional bacteriological conclusions confirmed or modified by topographical observations	Provisional bacteriological & topographical conclusions confirmed or modified by epidemiological & administrative considerations
I A water showing no evidence (bacteriologically) of objectionable contamination	No <i>B. coli</i> in 100 c.c.	For example:— (1) <i>Neutral-red</i> broth test—Greenish-yellow fluorescence (48 hrs. at 37° C.) (2) <i>Lactose peptone</i> test—Gas and acid production (48 hrs. at 37° C.) (3) <i>Indol</i> test—Indol in broth cultures (5 days at 37° C.) (4) <i>Litmus milk</i> test—Acid clotting of milk (5 days at 37° C.)	For example:— Dilution; set of the titides; set of the prevailing winds; float experiments; rate of flow; distance; time interval, etc.	For example:— Questions of practicality; whether the contaminating material is likely to have a high or a low enteric moribific value; past epidemiological experience in circumstances broadly parallel, etc. etc.
II A water showing appreciable, although slight, evidence (bacteriologically) of objectionable contamination	<i>B. coli</i> present in 100 c.c., none in 10 c.c.	Of course, the more tests applied the better, but the above are all tests of known value		
III A water showing definite signs (bacteriologically) of pollution, and therefore to be viewed with some degree of suspicion	<i>B. coli</i> present in 10 c.c., none in 1 c.c.	[As regards tests (1), (2), (3) and (4), my work for the Local Government Board on the <i>B. coli</i> of recently voided normal human faeces shows that of 101 <i>B. coli</i> , 98, 92, 98, and 92 per cent. respectively yielded positive results to one or other test. As regards all four tests (taken in conjunction) 85 per cent. yielded positive results.]		
IV A water showing such obvious signs (bacteriologically) of objectionable pollution as to be condemned on the basis of results	<i>B. coli</i> present in 1 c.c., none in 0.1 c.c. (1 not 10)			
V	<i>B. coli</i> present in 0.1 c.c. None in 0.01 c.c. (10 not 100)			
VI	<i>B. coli</i> present in 0.01 c.c., none in 0.001 c.c. (100 not 1000). [Primary standard for sewage effluents; non-drinking-water streams]			
VII	<i>B. coli</i> present in 0.001 c.c., none in 0.0001 c.c. (1000 not 10,000). [Secondary standards for sewage effluents; non-drinking-water streams]			

* It must, of course, be definitely understood that I am not prepared to say that a water of Class III, II, or even I is necessarily always "safe," much less that a water of Class IV—VII has a definite "disease value."

† This does not mean necessarily administrative practical or legislative condemnation, but rather that the evidence of pollution is sufficiently defined to merit objection from the bacteriologist's point of view.

N.B. The *B. enteritidis sporogenes* test is not dealt with here. Perhaps the safest standard to snowest is that a water should

sea-water, and, indeed, water and liquids in general, may be divided *broadly* into nine classes by means of the *B. coli* test¹.

4. This division of waters into classes does not involve necessarily the difficult question of absolute standards, but it may be convenient to condemn or object to a water of the fourth class (+ 1, - 1 c.c.); regard with some degree of suspicion a water of the third class (+ 10, - 1 c.c.); consider not wholly free from evidence of probably objectionable pollution a water of the second class (+ 100, - 10 c.c.); and unconditionally on the basis of results approve a water of the first class (negative 100 c.c.).

5. *B. coli*, artificially added to unpolluted sea-water and to tap-water speedily loses its vitality or, at all events, becomes greatly diminished in number under laboratory conditions of experiment. The continued persistence of *B. coli* in *any number* in estuarial waters may be traced to continuous excremental pollution and the presence of unoxidised organic pabulum in the water.

6. Neither the epidemiologist, nor the topographer, nor even the bacteriologist can assign a definite "disease value" to a given pollution.

7. Conclusions as regards the *degree* of potential danger to health arising from sewage pollution must, if sound, be based consciously or unconsciously on an assumed or real knowledge of the amount of fresh sewage matters present in the water, and more particularly on the number of living microbes of *recent* intestinal origin.

8. There appear to be three ways of estimating *degrees of pollution*. The topographical or inferential method²; the chemical or indirect (*qua* microbes) but broadly useful method³; and the bacteriological or seemingly most direct method⁴.

¹ Negative 100 c.c.; +100, -10 c.c.; +10, -1 c.c.; +1, -1 c.c.; +1, -01 c.c.; +01, -001; +001, -0001; +0001, -00001; +00001. First, second, third, fourth, fifth, sixth, seventh, eighth, and ninth classes respectively. It is obvious that these classes may be subdivided further, but whether or not this is desirable at present is a moot point. For example, we may subdivide the second class according to whether a positive result is yielded with 90, 80, 70, 60, 50, 40, 30, or 20 c.c. of the sample. Such subdivision might be expressed by the term sub-class, 1, 2, 3, 4, 5, 6, 7, 8, or 9 respectively. The same principle applies to the other classes.

² In the sense indicated here the topographical method theoretically is mainly speculative in character. But having regard to the breadth of its operations, it may be and indeed is actually of signal and indispensable value in practice.

³ The chemical method is a definite and extremely accurate method, and although indirect in character, may, within certain limits and in certain directions, yield most valuable results.

⁴ The bacteriological method is direct *qua* numerical estimation of intestinal microbes, but indirect as regards disease-producing bacteria. Although seemingly the best method, and extremely delicate, it is nevertheless to be thought of as of relative, not absolute, value.

9. In all but obvious cases of contamination *degrees* of pollution need to be measured by the bacteriologist, *who should interpret his results in the light of local observations and epidemiological considerations.*

10. However well-balanced the representations of the topographerist may be as regards estuarial pollution, they are expressions of opinion involving in large measure the personal equation, and if uninfluenced by quantitative and qualitative bacteriological data, may be lacking in comparative value, and may therefore be inconclusive.

IV. The Bacteriological Examination of Samples of Water and of Oysters obtained from the Helford and Penryn Rivers (Cornwall).

This report deals with the quantitative and qualitative bacteriological examination of water and oysters obtained from two rivers in Cornwall, not far distant from each other topographically, but widely different as regards their local surroundings.

The Helford River traverses a sparsely-populated district, and ranks as one of the purest localities in England for the growth and fattening of oysters.

The Penryn River is polluted, and its oyster layings lie under the ban of suspicion.

It is beyond question that the Helford River, from the point of view of the topographerist and the epidemiologist, would be approved. It is equally certain that the Penryn River, on topographical and epidemiological grounds, would be regarded with great suspicion, if not condemned.

The primary object of this investigation was to compare, bacteriologically, the water and oysters from these two rivers¹.

The results may be briefly summarised as follows :

¹ It is essential to note that in my detailed report to the Commission full details are given in each and every case of the biological characters of the coli-like microbes isolated in pure culture from both the waters and the oysters. It may be said, however, that the samples of Helford water and oysters (as well as the samples of Penryn water and oysters) were found to contain *typical B. coli* (on the basis of the tests employed) in greater or less number. The matter is largely one of proportion.

Helford Water.

<i>B. Coli</i> or coli-like Microbes			<i>B. enteritidis sporogenes</i>
100 c.c.	10 c.c.	1 c.c.	10 c.c.
Positive result	Positive result	Positive result	Negative result
28 %	40 %	32 %	100 %

*Helford Oysters.**B. coli* test:

1 out of 25 (4 %)	contained	1 <i>B. coli</i> or coli-like microbes	per oyster.
5 " (20 %)	"	10 " " "	" "
16 " (64 %)	"	100 " " "	" "
3 " (12 %)	"	1000 " " "	" "

B. enteritidis sporogenes test:

Less than 10 spores of *B. enteritidis sporogenes* per oyster, 16 out of 25, 64 per cent.

8 out of 25 contained 10 but less than 100, 32 %.

1 " 25 " 100 " " 1000, 4 %.

Penryn Water.

<i>B. coli</i> or coli-like microbes				<i>B. enteritidis sporogenes</i>	
10 c.c.	1 c.c.	1 c.c.	01 c.c.	10 c.c.	10 c.c.
Positive result	Positive result	Positive result	Positive result	Positive result	Negative result
16 %	36 %	44 %	4 %	56 %	44 %

*Penryn Oysters.**B. coli* test:

1 out of 25 (4 %)	contained	100 <i>B. coli</i> or coli-like microbes	per oyster.
13 " (52 %)	"	1000 " " "	" "
11 " (44 %)	"	10,000 " " "	" "

B. enteritidis sporogenes test:

3 out of 25 (12 %)	contained	10 spores of <i>B. enteritidis sporogenes</i>	per oyster.
20 " (80 %)	"	100 " " "	" "
2 " (8 %)	"	1000 " " "	" "

The following division of oysters into classes, with tentative standards for comparative purposes, may be of interest to fellow-workers (Table II. p. 182).

TABLE II. *Bacteriological Grouping of Oysters in Classes with Tentative Standards for Comparative Purposes.*

CLASS *	Standard based on numerical abundance of <i>B. coli</i> (or non-liquefying, gas-forming coliform-like microbes), in the whole contents of the oyster shell (i.e. liquor, body and interior juices of the oyster)	Numerical standard confirmed or modified according to response of the coliform microbes in pure culture to certain well-known biological tests	Provisional bacteriological conclusions confirmed or modified by topographical observations	Provisional bacteriological and topographical conclusions confirmed or modified by epidemiological and administrative considerations
I An oyster showing no evidence (bacteriologically) of objectionable contamination	No <i>B. coli</i>	For example:— (1) <i>Neutral-red broth test</i> —Greenish-yellow flocculence (48 hours at 37° C.) (2) <i>Lactose peptone test</i> —Gas and acid production (48 hours at 37° C.) (3) <i>Indol test</i> —Indol in broth cultures (5 days at 37° C.) (4) <i>Litmus milk test</i> —Acid clotting of milk (5 days at 37° C.) Of course, the more tests applied, the better, but the above are all known tests of value	For example:— Dilution: set of the tubes; prevailing winds; float experiments; time interval; distance, etc., etc.	For example:— Questions of practicability; whether the contaminating material is likely to have a high or a low enteric moribund value; past epidemiological experience in circumstances broadly parallel, etc., etc.
II An oyster showing appreciable, although slight, evidence (bacteriologically) of objectionable contamination	1 <i>B. coli</i> per oyster†			
III An oyster, showing definite signs (bacteriologically) of pollution & therefore, possibly to be viewed with some degree of suspicion	10 <i>B. coli</i> per oyster†			
IV An oyster showing such obvious signs (bacteriologically) of pollution as to be condemned on a stringent standard	100 <i>B. coli</i> per oyster†			
V An oyster showing such unmistakable evidence (bacteriologically) of pollution as to be condemned on the basis of results §	1000 <i>B. coli</i> per oyster†			
VI An oyster showing such gross evidence (bacteriologically) of contamination as to be outside the pale of recognition.	10,000 <i>B. coli</i> per oyster†			

* It must, of course, be definitely understood that it cannot be said either that oysters of Class II or even Class I are necessarily always safe, or that oysters of Class III—VI have a definite "disease value."

† To obtain approximate results *per c.c.* of oyster, divide the foregoing figures by ten.

§ This does not mean administrative, practical, or legislative condemnation, but only that the evidence of pollution is sufficiently defined to merit objection from the bacteriologist's point of view.

|| The foregoing results (1) (2) (3) and (4) may work for the Local Government Board on the *B. coli* of recently-voided normal human faeces shows that 100 *B. coli*, 88, 92, 98, and 99 per cent. yielded positive results in each instance to one or other test. As regards all four tests (taken in conjunction) 85 per cent. yielded positive results.

As regards the *B. enteritidis sporogenes* test, a lenient standard would seem to be less than 100 spores of this anaerobe *per oyster*. A more stringent standard would be less than 10 spores *per oyster*.

Conclusions.

On the one hand, the water of an estuarial river, such as the Helford River, which, on topographical grounds, has been, and would still be considered eminently well suited for the breeding, growth, and fattening of oysters for market, may contain *B. coli* or coli-like microbes, in 100 c.c. (28 %), in 10 c.c. (40 %), or in 1 c.c. (32 %). As regards *B. enteritidis sporogenes* all the samples yielded a negative result when using for cultural purposes 10 c.c. of the water.

Oysters obtained from the Helford River contained *B. coli* or coli-like microbes in greater number than did comparable quantities of the surrounding water: 1,000 per oyster, about 100 per c.c. of oyster (12 %); 100 per oyster, about 10 per c.c. of oyster (64 %); 10 per oyster, about 1 per c.c. of oyster (20 %); 1 per oyster (4 %). As regards spores of *B. enteritidis sporogenes*, the results were:—100 per oyster, about 10 per c.c. of oyster (4 %); 10 per oyster, about 1 per c.c. of oyster (32 %); less than 10 per oyster, *i.e.*, less than 1 per c.c. of oyster (64 %).

On the other hand, the water of an estuarial river such as the Penryn River, which on topographical grounds has been rightly considered dangerous *qua* oyster layings, but which *qua* bacteriological facts does not abound in microbes of intestinal origin, yielded, as regards *B. coli* or coli-like microbes, the following results:—100 per c.c. (4 %); 10 per c.c. (44 %); 1 per c.c. (36 %); + 10 c.c. (16 %). As regards the spores of *B. enteritidis sporogenes*, 56 % yielded a positive, and 44 % a negative result when using 10 c.c. for cultural purposes. The Penryn water was more than ten times as impure bacteriologically as the Helford water.

Oysters obtained from the Penryn River contained *B. coli* or coli-like microbes in much greater number than did comparable quantities of the surrounding water: 10,000 per oyster, about 1000 per c.c. of oyster (44 %); 1000 per oyster, about 100 per c.c. of oyster (52 %); 100 per oyster, about 10 per c.c. of oyster (4 %). As regards spores of *B. enteritidis sporogenes*, the results were:—1000 per oyster, about 100 per c.c. of oyster (8 %); 100 per oyster, about 10 per c.c. of oyster (80 %); 10 per oyster, about 1 per c.c. of oyster (12 %).

The Penryn oysters were therefore about 100 times more impure than the Helford oysters.

If the worst results from the "good" place (Helford) are to be

compared with the best results from the "bad" place (Penryn), assistance from the bacteriologist in determining the *status* of oysters or water is not to be looked for. On any other basis of comparison the contrast is most striking, and the results, in my opinion, indicate that the *quantitative* and *qualitative* bacteriological testing of oysters and estuarial waters may prove of great practical value in cases of doubtful pollution.

Neither oysters nor water are to be condemned on bacteriological grounds, unless the number of objectionable microbes exceeds what may be termed a "permissible limit of biological impurity." But where the line should be drawn remains to be determined.

Waters and oysters have been arranged in classes (*see* Table II.) according to the results of the *B. coli* test, and although this does not necessarily involve the acceptance of standards it may be convenient to adopt certain standards *solely for comparative purposes*.

For example :—To object to (1) a water containing *B. coli* in 1 c.c., or the spores of *B. enteritidis sporogenes* in 10 c.c.; and (2) to reject oysters containing 1000 (lenient standard) or 100 (stringent standard) *B. coli*; or 100 (lenient standard) or 10 (stringent standard) spores of *B. enteritidis sporogenes* respectively per oyster; *subject always to the examination of a number of samples, and to the interpretation of the results of the B. coli test in the light of a knowledge of the biological attributes of these microbes.*

Applying these tentative standards to the Helford and Penryn results, and including, *for the purposes of a broad summary*, "coli-like microbes," the following results are obtained :—

	Water			
	Penryn		Helford	
	Passed	Condemned	Passed	Condemned
<i>B. COLI</i> OR COLI-LIKE MICROBES : (Standard negative result, 1 c.c.)	16 %	84 %	68 %	32 %
<i>B. ENTERITIDIS SPOROGENES</i> TEST : (Standard negative result, 10 c.c.)	44 %	56 %	100 %	none
	Oysters			
	Penryn		Helford	
	Passed	Condemned	Passed	Condemned
<i>B. COLI</i> OR COLI-LIKE MICROBES : <i>Lenient Standard</i> (less than 1000 per oyster, or 100 per c.c. of oyster)	4 %	96 %	88 %	12 %
<i>Stringent Standard</i> (less than 100 per oyster, or 10 per c.c. of oyster)	none	100 %	24 %	76 %
<i>B. ENTERITIDIS SPOROGENES</i> TEST : <i>Lenient Standard</i> (less than 100 per oyster, or 10 per c.c. of oyster)	12 %	88 %	96 %	4 %
<i>Stringent Standard</i> (less than 10 per oyster, or 1 per c.c. of oyster)	none	100 %	64 %	36 %

Method adopted in Examining Oysters Bacteriologically.

Cleansing of the Oysters:—

The outside of the oyster shells was well scrubbed with soap and water, and cleansed as thoroughly as possible under clean running water; the shells were then well washed in running *main* water, and finally with sterile water.

Cleansing of the Hands:—

The hands of the experimenter were thoroughly cleansed with a hard scrubbing brush, soap, and water, then rinsed first with 1 in 1,000 corrosive sublimate solution, and finally with sterile water.

Subsequent procedure:—

The oysters were laid out upon a sterile towel, the flat shell uppermost. They were opened in this position with a sterile knife, held in the right hand, while they were held in position with a corner of the sterile cloth grasped in the left hand. Great care was taken to avoid any loss of the liquor. This liquor in the shell was poured into a sterile 1000 c.c. cylinder, the oyster was then partly cut up with sterile scissors, and the liquor thus freed also allowed to run into the cylinder; finally, the oyster was cut up into small pieces, and added to the cylinder. *Ten oysters were thus treated in each experiment.* The volume of oyster + oyster liquor was *read off*, and usually varied between 80 and 120 c.c., so that the oysters, being of *medium* size and containing a medium amount of liquor, 100 c.c. might be considered a fair average¹ of the total shell contents of ten oysters. Sterile water was then poured into the cylinder up to the 1000 c.c. mark, and the whole well stirred with a sterile rod.

The following amounts of this liquid were taken for cultural purposes (primary cultures):—

¹ This average has been used generally as a convenient basis for calculating the relation between the number of bacteria per c.c. in the oyster and the water over the oyster layings. It is, however, an under-estimate of the bulk of many oysters. It must be remembered that the bacteria are not uniformly distributed within the contents of the oyster shell. No doubt the liquor, alimentary tract and perhaps the gills harbour most of the microbes, and the tissue of the body of the oyster may be, relatively speaking, sterile.

Culture A 100 c.c. = contents of 1 oyster.

- „ B 10 c.c. = „ $\frac{1}{10}$ „ multiply by 10.*
 „ C 1 c.c. = „ $\frac{1}{100}$ „ „ „ 100.*
 „ (1) 1 c.c. of $\frac{1}{10}$ dilution (.1 c.c.) = $\frac{1}{1000}$ oyster, multiply by 1000.*
 „ (2) 1 c.c. of $\frac{1}{100}$ dilution (.01 c.c.) = $\frac{1}{10000}$ oyster, multiply by 10,000.*
 „ (3) 1 c.c. of $\frac{1}{1000}$ dilution (.001 c.c.) = $\frac{1}{100000}$ oyster, multiply by 100,000.*
 „ (4) 1 c.c. of $\frac{1}{10000}$ dilution (.0001 c.c.) = $\frac{1}{1000000}$ oyster, multiply by 1,000,000.*

* To obtain the number of bacteria, e.g. *B. coli*, per oyster. (Divide the figures thus obtained by 10, if the results per c.c. of oysters are wanted.)

These amounts A, B, C, 1, 2, 3, 4 were used for the examination for *B. coli* (primary cultures), and the amounts B, C, 1, 2, 3 for the *B. enteritidis sporogenes* test. Experience has shown that it is best to make the primary cultures in triplicate. Then if, as regards the *B. coli* test, a sugar medium is employed, at least two out of the three primary cultures should form acid and gas to allow of a *preliminary numerical* diagnosis being made. Similarly, in respect of the *B. enteritidis sporogenes* test, the “enteritidis” change should occur in at least two out of the three anaerobic milk cultures to merit a positive result being recorded.

The subsequent procedure, so far the *B. coli* test was concerned, was the isolation of *B. coli* or coli-like microbes from the primary cultures, by means of secondary gelatine plate cultures, followed by study of the isolated microbe in the *pure state* in following media :—

Gelatine “shake” cultures (for “gas” formation); Broth cultures (for indol formation); Litmus milk cultures (for acid and clotting); Lactose peptone cultures (for acid and gas); neutral-red broth cultures (for greenish-yellow fluorescence).

As regards *B. enteritidis sporogenes*, the inoculation of animals was not practised, so that the results must be interpreted in the sense that a positive result means the “enteritidis change” in anaerobic milk culture without proof of virulence.

The chief advantages of this method are as follows :—

1. It is a *definite quantitative* method, succeeded by qualitative records.
2. It gives the average volume of the whole contents of the oyster shell.
3. It yields results based on collective examination of ten oysters.
4. It includes the examination of the entire contents of the shell,

not of a fraction either of the liquor or the gastric or intestinal juice, or of the mixture of these liquids.

5. The results can be stated as number of bacteria, either per oyster, or per c.c. of oyster.

An alternative Quantitative Method for the Bacteriological Examination of Oysters.

An alternative method for the bacteriological examination of oysters may be given here, although the routine work was carried out by the foregoing method.

The oysters are cleaned and opened, with the precautions already noted. Then the body of the oyster is cut into small pieces with sterile scissors; this process should be carried out in such a way as to ensure the thorough mixture of the gastric juice of the oyster and the liquor. The oyster, meanwhile, is carefully held with the concave shell downwards and the flat shell bent back or altogether removed. To examine the liquid contents of the shell without this preliminary step may partake of the nature of the examination of the last sample of sea-water imbibed by the oyster before finally closing its shell. Indeed, the experiments detailed elsewhere seem to indicate that *per unit of volume* the gastric juice of the oyster may be more impure bacteriologically than the oyster liquor.

The next step is to withdraw 1 c.c. of the oyster mixture with a sterilised 1 c.c. pipette and add it to 9 c.c. of sterile water in a test tube (dilution 1). 1 c.c. of dilution (1) is used to inoculate a second tube containing 9 c.c. of sterile water (dilution 2). 1 c.c. of dilution (2) is used to inoculate a third tube containing 9 c.c. of sterile water (dilution 3), and so on to further dilutions if necessary. Primary cultures, using in each instance 1 c.c., are next made severally from dilutions (1) (2) (3), corresponding to $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$ c.c., respectively, of the oyster mixture.

Further, 1 c.c. of the oyster mixture direct, that is without any dilution, is used to make another primary culture. From these primary cultures, after incubation for two days at 37° C., secondary plate cultures are made, and from these plates the coli-like colonies are subsequently picked out and studied in pure culture in various media.

The above procedure applies to the *B. coli* test, but it is obvious

that the same dilutions might also be employed for the *B. enteritidis sporogenes* test.

The above method answers fairly well if the oysters contain a sufficient volume of liquor, and if conclusions are based on the examination of at least 10 oysters. Nevertheless, this method is not free from certain objections. The volume of liquor in oysters varies enormously, ranging from as little as 0.1 c.c. to over 10 c.c., and the results might naturally vary in corresponding degree. Sometimes the amount of liquor is too small to allow more than one culture being made, even if that end be achieved, at other times it is so large as to suggest that sea-water is diluting the bacterial contents of the oyster itself to a considerable extent. At all events, it is obvious that if the liquid contents of the shell may vary one hundred times, it is difficult to ensure that comparative quantitative records are always obtained.

V.

Appendices A to L.

Results of a number of separate bacteriological observations bearing on the general question of the pollution of estuarial waters and shell-fish.

APPENDIX A.

Results of the Bacteriological Examination of samples of Sea-Water, Estuarial Water, water over shell-fish layings, etc. Collected for the most part during the visits paid by the Commission to various centres of the Oyster Industry.

Generally speaking the bacteriological results were broadly parallel with the topographical surroundings of the places whence the samples were derived.

With a view of rendering a general survey of all the results as regards *B. coli* comparatively easy, the samples have been grouped (irrespective of the biological attributes of the coli-like microbes) in separate classes (— 10 c.c.; + 10 c.c., — 1 c.c.; + 1 c.c., — .1 c.c.; + .1 c.c., — .01 c.c.; + .01 c.c., — .001 c.c.; + .001 c.c., — .0001 c.c.; + .0001 c.c., — .00001 c.c.; + .00001 c.c.) as follows:—

B. coli test.—Samples yielding a negative result with 10 c.c. of the sample.

No.

5. Gutner Creek, oyster pond.
- B. Solent, midway between Ryde and Southsea piers.
17. Crouch River, 1 mile above Burnham.
22. Crouch River, fattening beds at mouth of river.
25. Roach River, Barling Creek, top of fishery.
26. Roach River, storage pit at Poole Creek.
32. Blackwater, Strood Channel, opposite Victory Inn.
33. " " " 100 yards higher up.
1. Hunstanton, high water, $\frac{1}{4}$ mile from land.
2. " low " " "
3. " high " $\frac{1}{2}$ mile west of pier.
4. " low " " " "
5. " " " " from sewage outfall.
6. " high " 1 mile from shore opposite sewage works.
61. " Sea pool over mussel beds, 1 mile N.W. pier.
62. The Wash, about $\frac{2}{3}$ flood tide, near Roaring Middle.
1. Firth of Forth at Gullane, shore sample.
2. " " " " "
3. " " " " "
4. " " " " "
5. " " " " "
6. Ribble estuary at Lytham, boat sample, high water.
36. North Sea, opposite Harwich, some miles out to sea.
1. " " off Flamboro' Head, 5 miles out.
- A. English Channel, midway between Newhaven and Dieppe.

B. coli test.—Samples yielding a positive result with 10 c.c., but a negative result with 1 c.c. of the sample.

No.

2. Langston Channel, flood tide, near ferry.
3. Solent Water, shore sample, opposite Grand Hotel.
4. North Saltern Creek, near mouth of Emsworth Channel.
- A. Langston Channel, flood tide, near ferry.
23. Crouch River, at Holywell, 3 parts flood tide.
24. Roach River, at White House fattening beds, high water.
29. Pyfleet Channel, opposite packing house, $\frac{2}{3}$ flood tide.
30. Pyfleet oyster pit, No. 23, near packing house.
35. Pyfleet Channel, head of channel, about high tide.
34. West Mersea, Mr Bean's oyster pit.
- 34A. " " but mud stirred up.
51. Teign River under Shaldon Bridge, $\frac{2}{3}$ flood tide.
53. " " opposite gas works, $\frac{5}{6}$ flood tide.

No.

60. Brancaster Staith, mussel pit.
6. Firth of Forth, at Gullane, shore sample.
1. Ribble estuary at Lytham, high water, 1 mile from pier.
3. " " " " " " $\frac{1}{2}$ " " "
10. Bosham, oyster pit.
15. Southwick, one of Brazier's oyster ponds, low water.
63. Mouth of Witham River, dredged sample of mud and water.

The last three samples yielded a negative result with 1 c.c. of the sample, but no 10 c.c. cultures were made.

B. coli test¹.—Samples yielding a positive result with 1 c.c., but a negative result with $\frac{1}{10}$ c.c. (1 not 10 in 1 c.c.).

No.

6. Langston Channel, near high tide, near Hayling fishery.
7. Emsworth longshore oyster pit (Kennet).
11. Chichester Channel, Del Quay, about high water.
13. Southwick, one of Brazier's oyster ponds, low water.
27. Brightlingsea Creek. over private layings, 2 hours flood.
28. Colne Channel, extreme point of fishery, 2 to 3 hours flood.

B. coli test.—Samples yielding a positive result with $\frac{1}{10}$ c.c., but a negative result with $\frac{1}{100}$ c.c. (10 not 100 in 1 c.c.).

No.

8. Emsworth longshore oyster pit (Foster).
14. Southwick Channel, opposite Brazier's ponds, $\frac{1}{4}$ flood.
20. Burnham, pile house, Gutter Creek.
54. Teign River, between Combe Cellars and Shaldon Bridge, $\frac{5}{8}$ flood.
56. " " mussel bank above gas works, $\frac{5}{8}$ ebb tide.
58. " " underneath Shaldon Bridge, low water.
59. " " opposite Devon trading wharf, low water.
4. Ribble estuary at Lytham, off pier, low water.
5. " " " " " "
16. Sea-water off Brighton, 300 yards east of Portobello outfall.

¹ According to the tentative standard of a negative result with 1 c.c. (see Division III.), all the foregoing samples would be passed, and all the following samples rejected bacteriologically. That is, if the numerical results, as regards *B. coli* and coli-like microbes, be considered independently of the biological attributes of such microbes.

B. coli test.—Samples yielding a positive result with $\frac{1}{100}$ c.c., but a negative result with $\frac{1}{1000}$ c.c. (100 not 1000 in 1 c.c.).

No.

9. Emsworth Channel, 7 yards below sewer outfall, $\frac{1}{2}$ flood tide.
12. Shoreham Channel, just above Norfolk Bridge, near low tide.
52. Teign River, about 2 miles above Shaldon Bridge by Combe Cellars, $\frac{2}{3}$ flood tide.
55. Teign River, between Combe Cellars and Shaldon Bridge, $\frac{5}{8}$ ebb tide.
57. „ „ opposite gas works, low water.
2. Ribble estuary at Lytham, $\frac{1}{2}$ mile south of pier, low water.

B. coli test.—Samples yielding a positive result with $\frac{1}{1000}$ c.c., but a negative result with $\frac{1}{10000}$ c.c. (1000 not 10,000 in 1 c.c.).

No. 18. Burnham, fine bed effluent.

B. coli test.—Samples yielding a positive result with $\frac{1}{10000}$ c.c., but a negative result with $\frac{1}{100000}$ c.c. (10,000 not 100,000 in 1 c.c.).

No samples come under this category, but the heading is retained for the sake of uniformity.

B. coli test.—Samples yielding a positive result with $\frac{1}{100000}$ c.c. (at least 100,000 per c.c.).

No.

19. Burnham, combined effluent.
21. Burnham, mixed effluent and sea-water at sluice.
31. Brightlingsea, tank effluent, Ives' patent.
64. Hunstanton, sewage effluent, at outfall.

APPENDIX B.

Experiments dealing with the Bacteriological Examination of Deep Sea Oysters and surface samples of Sea-Water collected over the Oysters.

The results show that in deep sea oysters derived from deep sea-water remote from sewage pollution *B. coli* and coli-like microbes and also the spores of *B. enteritidis sporogenes* are either absent or, at all events, seldom detectable. The same is true of the surface water over such oysters.

It is nevertheless true that in shallow water near the shore around our English coast, in situations practically available and well suited

for the growth and fattening of high class oysters, it may be and indeed would seem to be impossible to obtain similar bacteriological results.

Yet it is necessary to start with a basis, and the basis in the present instance is that *B. coli* and *B. enteritidis sporogenes* seemingly form no essential part of the bacterial flora of pure sea-water, and that they have no part in the economy of the oyster.

Without going into the experiments in detail it may be said that certain of the experiments indicate that water from the open sea collected about 9 miles from the Norfolk coast may be *at least one-hundred million times purer* (qua bacteriological facts) *than a sewage effluent of average quality; from 10 to 100 times purer than Whitstable sea-water; from 100 to 10,000 times purer than West Mersea sea-water; from 10 to 1,000 times purer than Crouch River water; from 100 to 1,000 times purer than Roach River water; and from 1,000 to 10,000 times purer than Pyfleet water derived from the neighbourhood of the oyster layings in these localities respectively.*

With Deep Sea Oysters the result was frequently totally negative as regards the presence of coli-like microbes of any sort. For example, in one experiment a total of 57 c.c. of liquor derived from five oysters contained no coli-like microbes.

APPENDIX C.

Results of the Bacteriological Examination of Samples of Whitstable, West Mersea, Crouch, Roach, and Pyfleet oysters and corresponding samples of water.

Two hundred oysters were examined altogether, in four separate batches of ten oysters from each of the foregoing five localities. Twenty corresponding samples of water collected over the layings were examined altogether; four from each of the above-named five localities.

The Whitstable and Pyfleet oysters are known all over the world, and the West Mersea, Crouch and Roach oysters are probably no whit inferior in quality. No outbreak of enteric fever has ever, it is believed, been traced to the consumption of oysters from these layings. At the great annual Colchester oyster feast, tens of thousands of Pyfleet oysters are consumed in one day. The general topographical surroundings of these oyster layings have been either unconditionally approved or the layings considered so far removed from *recent* sewage

pollution as to be practically safe¹. These practical considerations should be borne in mind in scrutinising the results of the bacteriological examinations.

The chief results may be summarised briefly as follows :

Waters.

Number of coli-like microbes considered independently of their biological attributes.

[Tentative standard, none in 1 c.c.]

	+ 100 c.c. No. of samples	+ 10 c.c. No. of samples	+ 1 c.c. No. of samples	+ $\frac{1}{10}$ c.c. No. of samples
Whitstable water	1	3	—	—
West Mersea water	—	2	1	1
Crouch river	2	1	1	—
Roach river	—	2	2	—
Pyfleet water	—	—	1	3

B. enteritidis sporogenes :

[Tentative standard, negative result 10 c.c.]

	Negative 10 c.c. No. of samples	+ 10 c.c. No. of samples	+ 1 c.c. No. of samples
Whitstable water	3	1	—
West Mersea water	4	—	—
Crouch river	4	—	—
Roach river	4	—	—
Pyfleet water	2	1	1

Oysters.

Number of coli-like microbes² considered independently of their biological attributes.

[Tentative standards :—less than 1000 (lenient standard); less than 100 (stringent standard) per oyster.]

	10 per oyster No. of samples	100 per oyster No. of samples	1000 per oyster No. of samples
Whitstable oysters	3	1	—
West Mersea oysters	1	2	1
Crouch oysters	1	2	1
Roach oysters	—	4	—
Pyfleet oysters	—	3	1

¹ It must, of course, be understood that throughout this article no personal responsibility is incurred for topographical expressions of opinion as regards the purity or otherwise of oyster layings. The rule has been to accept the opinions of the highest authority on the subject, as if they were final, for the purposes of comparison with the bacteriological results.

² Within the limits of this article it is impossible to consider the “modifications of test readings” suggested in my detailed report to the Commission. It may be said, however, that the samples both of water and of oysters obtained from these layings occasionally, if not uniformly, contained *typical B. coli* (on the basis of the tests employed) in greater or less number. The matter is largely one of proportion.

B. enteritidis sporogenes test:

[Tentative standard:—less than 100 spores (lenient standard); less than 10 spores (stringent standard) per oyster.]

	Less than 10 per oyster No. of samples	10 per oyster No. of samples	100 per oyster No. of samples
Whitstable oysters	—	1	3
West Mersea oysters	3	1	—
Crouch oysters	—	3	1
Roach oysters	2	2	—
Pyfleet oysters	1	3	—

If the Whitstable, West Mersea, Crouch, Roach and Pyfleet layings are to be accepted as pure on topographical grounds it is obvious that a "certain degree of biological impurity" must be accepted as permissible if oyster culture is to be encouraged around our English shores. But where the line should be drawn is matter for conjecture.

APPENDIX D.

Experiments undertaken to ascertain whether *B. coli* or coli-like microbes can be practically always isolated from a mixture of ten oysters derived from layings which, judged from the topographical point of view, would be considered either above suspicion or at least reasonably secure from pollution; and also to ascertain whether such microbes can usually be isolated from each individual, or, at all events, from a majority of the ten oysters experimentally tested.

The Helford, Whitstable, West Mersea, Crouch, Roach and Pyfleet oyster layings have all been approved on topographical grounds. These layings are indeed considered to be, from the topographical point of view, among the purest (some of them perhaps *the purest*) layings around the *English Coast*.

To suggest therefore that the oysters from these layings are impure, in the sense that they are a source of danger to health, would be a grave step to take. It would be equivalent to implying that secure conditions of oyster culture in England are practically unattainable.

As a bacteriologist I offer no opinion as to the possibility or otherwise of oysters from these layings giving rise to epidemic disease.

Quantitative Results.

Estimated number of coli-like microbes *per c.c.* of oyster liquid (mixed liquid and juices of oyster).

Oyster	1	2	3	4	5	6	7	8	9	10
Helford	100	100	100	100	100	100	100	10	10	10
Whitstable	10	10	10	10	10	1	1	1	1	1
West Mersea	10	10	10	10	1	1	1	1	1	none
Crouch	10	10	1	1	1	1	1	1	1	1
Roach	10	10	10	10	10	10	1	1	1	none
Pyfleet	10	10	10	1	1	1	1	1	1	1

It is not suggested that these figures should be accepted as indicating the respective merits of the different oysters. The point of interest is that as regards 60 oysters (10 from each of six separate layings) the number of coli-like microbes corresponded :

In 7 oysters (11·6%) to one hundred	} coli-like microbes per c.c. of oyster liquid (mixed liquor and juices of oyster).
„ 23 „ (38·3%) „ ten	
„ 28 „ (46·6%) „ one	
„ 2 „ (3·2%) „ not any	

Many of these coli-like microbes were not identical in behaviour with typical *B. coli*, but the only point emphasized here is that practically all the oysters derived from certain of the *reputedly* purest layings in England could be regarded as containing from 1 to 100 coli-like microbes per c.c. of oyster liquid (mixed liquor and body juices).

Qualitative Results.

As regards a list of the biological characters of the *B. coli* contents of individual oysters, much depends on the amount of material used for cultural purposes, and also on the “chances” involved in the speculative choice of colonies for subculture. This being understood, it is justifiable, in relation to my thesis, to select the particular batch of ten oysters from each of the six layings which, when submitted to examination, yielded the most typical *B. coli*¹.

¹ It must be understood, definitely, that I have *not*, as regards any of the layings, selected oysters from *separate* batches, so as to make up a total of ten oysters, but have chosen the particular batch of ten oysters out of a series of batches of ten oysters yielding the most typical *B. coli*.

To express the results of subcultural tests the word "flaginac" will be used in the following sense :

fl	ag	in	ac
Indicates greenish-yellow fluorescence in neutral-red broth cultures.	Indicates acid and gas in lactose peptone cultures.	Indicates indol formation in broth cultures.	Indicates acidity and clotting of litmus milk cultures.

The word "flaginac" thus indicates that a microbe was indistinguishable, as regards the tests employed, from the typical *B. coli* of the human intestine. Whenever letters are placed in brackets this indicates an incomplete reaction. The absence of a character is expressed by the omission of the letters chosen to indicate that attribute.

Biological Attributes of the most typical Coli-like Microbes derived from each of 60 Oysters independently of their relative Abundance.

Oysters	1	2	3	4	5	6	7	8	9	10
Helford Exp. I.	Flag- inac	Flag- inac	Flag- inac	Flag- inac	Flag- inac	Flag- inac	Flag- inac	Flag- inac	Flag- inac	Flag- inac
Whitstable Exp. III.	"	"	"	"	"	"	"	"	Flag (ac)	Agac
West Mersea Exp. VI.	"	"	"	"	"	"	"	"	Flag- inac	Flag- inac
Crouch Exp. VIII.	"	"	"	"	"	"	"	"	"	Flag
Roach Exp. XI.	"	"	"	"	"	"	"	"	"	Flagin (ac)
Pyfleet Exp. XIII.	"	"	"	"	"	"	"	"	"	Flag- inac

It thus appears as regards 60 oysters (10 from each of six separate layings), all of which contained coli-like microbes, that the biological attributes of the microbes in question may be expressed as follows :—

56 oysters contained "flaginac" microbes					} That is, 93% of the oysters contained microbes indistinguishable, as regards the tests employed, from the typical <i>B. coli</i> of the human intestine.
1 oyster	"	flagin(ac)	"	"	
1 oyster	"	flag(ac)	"	"	
1 oyster	"	agac	"	"	
1 oyster	"	flag	"	"	

The inference is that practically all the best known English oysters are liable to contain, given a batch of ten oysters, coli-like microbes in each of the ten oysters (100 %), and that these coli-like microbes may be indistinguishable, as regards the tests employed, from the typical *B. coli* of the human intestine.

The matter may be regarded as one of *proportion*; and, in the circumstances, until the "permissible degree of biological impurity" can be defined, no English oysters should be condemned on bacteriological grounds alone, unless, indeed, the results indicate bacteriologically such *gross* pollution as to afford no possible room for doubt of their objectionable quality. This does not mean that the quantitative and qualitative bacterioscopic examination of oysters is unimportant. The contrary is my own view, but until knowledge is more complete, it would, I consider, be unjustifiable to damage an important industry by relying *exclusively* on the bacteriological facts, the *precise* interpretation to be placed on these facts remaining matter for speculation.

Meanwhile demonstration of the *mere presence* of *B. coli*, as the result of qualitative bacteriological analysis, even in 90 % of a batch of oysters from any layings around the English coast, would seem, in face of the above results, insufficient to condemn such layings.

APPENDICES E—K.

In *Appendix E* of the Report will be found the results of experiments designed to ascertain whether immersion for a considerable time of the bodies of oysters in strong germicidal solutions is to be relied on to destroy *B. coli* (or coli-like microbes); whether, that is, these microbes are or are not contained within the alimentary tract of the oyster.

APPENDIX F refers to experiments designed to ascertain whether or not prolonged washing of the bodies of oysters after removal from their shells frees such washed oysters of microbes of undesirable sort. The results show that neither prolonged washing of the bodies of polluted oysters nor immersion in germicidal solutions can be relied on to free such oysters from *B. coli* or coli-like microbes owing to the presence of these bacteria in the interior of the oysters.

APPENDIX G relates to a quantitative series of experiments to ascertain the relation between the biological composition of (1) the liquor and "washings" of the oyster and (2) the body of the oyster.

Without entering into detail it may be said that the results appear

to indicate that the "coli-yielding fraction" of the body of the oyster may contain *per unit of volume* a larger proportion of coli-like microbes than the liquor.

APPENDIX H contains: I. A series of experiments to ascertain whether *B. coli* (or coli-like microbes) are normally present in the stomach of the oyster.

II. A series of quantitative experiments to determine the number of *B. coli* (or coli-like microbes) present in the stomach juice of oysters.

Briefly stated, the results show that *B. coli* (or coli-like microbes) may not only be present in the alimentary tract of certain oysters but that the proportionate number of these undesirable bacteria may be very great.

APPENDIX I. Experiments designed to ascertain whether the *B. coli* met with within the shell of the oyster may have been derived from the exterior of the shell or from the manipulative procedure involved in the opening and examination of the shell contents.

The experiments clearly show that whatever precautions be taken as regards examination polluted oysters contain *within* their shells microbes of undesirable sort.

APPENDIX J. Experiments designed to ascertain whether or not polluted oysters re-laid in sea-water remote from sewage pollution can rid themselves within a reasonable time of microbes of undesirable sort (e.g. *B. coli*).

The results, on the whole, were disappointing but the subject merits a prolonged investigation.

APPENDIX K. Experiments designed to ascertain the length of time that *B. coli* or coli-like microbes can persist in oysters when the latter are separated from their natural environment and placed under artificial, and, if the term be admissible, "dry" conditions.

The results conclusively show that *B. coli*, originally present in oysters, *may* not lose its vitality for more than a week under the conditions of experiment. The assumption that *B. coli* *rapidly* perishes in oysters when the latter are separated from their natural environment is thus not borne out by the results of my analysis.

APPENDIX L.

The results of the bacteriological examination of oysters bought either in the market or at well-known fish shops or restaurants.

Anglo-Dutch, Whitstable, Portuguese, Blue Point, and Burnham

oysters were examined. They were bought at Sweeting's, Spiers and Pond's, Driver's, Scott's, and the Farringdon Market.

All the oysters contained coli-like microbes, and in the majority of instances, either in the last dilution yielding coli-like microbes, or in the last dilution-but-one microbes, were isolated, indistinguishable with regard to the tests employed from the *B. coli* of the human intestinal tract. Further, in the great majority of instances the *B. enteritidis sporogenes* test yielded positive results.

The results obtained would seem to indicate the danger of hastily condemning (on bacteriological grounds) oysters placed on the market for sale, for example:

(1) An oyster might have been derived from a relatively pure locality, but at the period when it was submitted to examination the few coli-like microbes originally present might conceivably for some reason or other have multiplied in the oyster.

(2) On the other hand, an oyster might have been derived from a polluted laying, but at the period when it was submitted to examination, the coli-like microbes originally present might conceivably for some reason or other have declined in number in the oyster.

(3) Previous to examination the oysters may have been kept under sanitary or insanitary conditions, placed in clean or dirty water, mixed or unmixed with meal or other substance.

Acknowledgement must be made of the large amount of work carried out by previous workers on this subject. Among the bacteriologists in this country who have devoted much time and attention to the subject the names of Boyce, Buchanan, Foulerton, Herdman, Hewlett, Klein, Lorrain Smith, McWeeney, Scholberg and Thresh must be mentioned. Recently Dr Eyre has published a preliminary note on the "Distribution of *B. coli*" which has an important bearing on the subject. Reference must also be made to a most instructive paper by Messrs Clark and Gage, the American bacteriologists¹. These two observers come to the conclusion that "the ability to demonstrate clearly the presence of a specific sewage organism, such as *B. coli*, is an invaluable aid in determining the question of purity or pollution." They, however, qualify this statement in the next sentence, as follows: "In many samples from polluted sources *B. coli* has not been found in

¹ "On the value of tests for bacteria of specific types as an index of pollution," by Messrs Clark and Gage. From the Thirty-fourth Annual Report of the State Board of Health for Massachusetts for 1902.

either shell water or intestine." Their explanation is that "among the many bacteria normally present or finding lodgement in the intestine or in the shell water, the stronger and more numerous species may evidently destroy the *B. coli* before laboratory examination is possible." I do not quite agree with this explanation and believe that failure to isolate *B. coli* in these cases arose not from the real absence of this microbe but to the presence of other bacteria in such abundance as to render the isolation of *B. coli* a matter of considerable difficulty.

The authors also examined (a) the intestines of shell-fish, (b) shell-fish liquor and (c) sea-water, the samples of shell-fish and sea-water being collected at varying distances from a sewer outfall. In all cases (a, b, c) *B. coli* was found in a larger percentage of samples collected $\frac{1}{2}$ to $\frac{3}{4}$ mile from the sewer outfall than in similar samples (a, b, c) collected 0 to $\frac{1}{8}$ mile from the source of contamination. The explanation again suggested is that *B. coli* is destroyed in polluted samples of water and shell-fish between the time of collection and of laboratory examination.

Whatever the true interpretation of these facts may be, it will not be disputed that the more *recent* and the more *gross* the pollution the greater is the element of potential danger to health likely to be. Hence if the bacteriologist is specially apt to encounter negative results in cases of gross and recent pollution the practical utility of his tests is open to question.

The results obtained by these observers were achieved by qualitative, not by combined quantitative and qualitative methods, and there is seemingly no record in their paper of the amounts of material submitted to cultural tests. If their investigations had been carried out on a combined quantitative and qualitative basis I believe their results would have shown that, on the average, the number of *B. coli* and of coli-like microbes in shell-fish and estuarial waters runs broadly parallel with the degree of pollution.

The section devoted to shell-fish forms but a relatively speaking unimportant portion (pages 18 to 20) of the entire report (36 pages) by Messrs Clark and Gage. Speaking of the report as a whole it may be said that the investigations cover much new ground, afford a fund of useful information and suggest many new lines of inquiry of an important kind.

AN IMPROVED METHOD OF MEASURING THE AMOUNT OF PRECIPITUM IN CONNECTION WITH TESTS WITH PRECIPITATING ANTISERA.

By GEORGE H. F. NUTTALL, M.A., M.D., PH.D.,
AND O. INCHLEY, M.A., M.B.

(*From the Pathological Laboratory, University of Cambridge.*)

FURTHER experience with the method of measuring the precipitum obtained when experimenting with precipitating antisera has shown that *finer* degrees of reaction cannot be satisfactorily determined by means of the apparatus described by Nuttall¹. In the latter apparatus capillary tubes were used in which to measure the precipitum therein deposited. It is true that capillaries as far as possible of the same calibre were used, nevertheless we have found that different readings were obtained when experimenting with tubes of varying calibre, variations evidently due to physical causes which prevent an equal degree of "packing" of the deposit in tubes of unequal width. We have modified the method after finding that calibrated tubes of uniform bore gave more constant results, and have devised a simple apparatus which greatly facilitates the making of measurements.

The Calibrated Tubes.

Several lengths of wide thermometer tubing are selected, the calibre being such that .05 c.c. of fluid occupies about 20 mm. of the bore of the tube. This size was found to be a convenient one when working with .5 c.c. of a 1 : 21 dilution of serum to which .1 c.c. of antiserum of average strength was added².

¹ Nuttall (5. iv. 1902), *Brit. Med. Journ.* vol. i, pp. 825—827, reprinted in Nuttall (1904), *Blood Immunity and Blood Relationship etc.* pp. 315—318.

² A fine piece of glass rod, drawn out so that it tapers gradually, serves as a convenient cone for measuring the calibres of the tubing.

The thermometer tube having the desired calibre is drawn out and cut in the form represented in Fig. 2, the uniform part of the tube (*D*) measuring about 11 cm., the tapered part (*F*) about 7 cm. so as to be conveniently introduced into the small test-tube (*E*) containing mercury, to the bottom of which it reaches. The tapered extremity moreover enables the operator to completely drain the contents of the small test-tubes in which precipitation has previously been allowed to take place. The object of the mercury in the small test-tube is to prevent the escape of the fluid (containing the precipitated matter in suspension), when the calibrated tube is stood upright. The tapered end of the tube should be as strong as possible and have a bore of about .5 mm.

Each tube is carefully calibrated, the lower mark *B* being on the shank just above the shoulder. The space between graduations *B* and *A* above represents .05 c.c. Each tube is marked with a number at *C*, sets of tubes being ordered according to the uniformity between the graduation marks *A* and *B*, the variations in calibre being noted.

Method of using the Tube.

Having added antiserum to serum dilution in the usual manner the mixture is shaken. The test-tubes are allowed to stand for 24 hours to permit the precipitum to accumulate at the bottom. (Further details will be found in the publications by Nuttall already referred to.) The greater part of the supernatant fluid is now drawn off, and the residue, together with the precipitum, is taken up into the calibrated tube. This is accomplished by holding the test-tube in the left hand, the tube in the right with its tapered extremity reaching to the bottom of the fluid in the test-tube, suction being gently applied by means of a piece of rubber tubing applied loosely to the end *C* of the calibrated tube. By applying the rubber tubing loosely a certain amount of air enters from without at the joint and gentle suction can be applied without fear of drawing up the fluid and precipitum suddenly into the rubber tubing thus vitiating the experiment. When the test-tube has been nearly emptied, the finger is applied at *C*, both tubes are inclined gradually, the finger regulating the inflow of the remaining fluid, until finally the former is on a higher level than the calibrated tube, into which the contents of the test-tube almost completely drain. The calibrated tube is now held horizontally, the finger applied to end *C* and the fluid allowed to flow along the tube,

by tilting it, until the lower meniscus is just above the graduation mark *B*.

The tube, held in the manner described, is now placed vertically, nozzle downward in the small test-tube containing mercury, and the tubes are placed on the rack, as shown in Fig. 1 (1). After standing

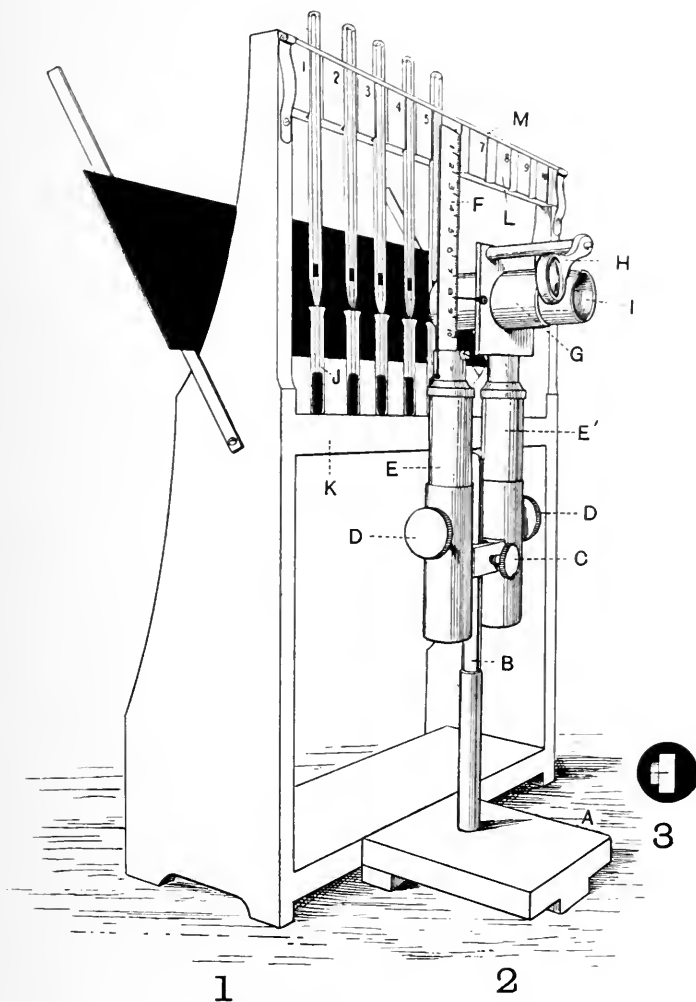


Fig. 1.

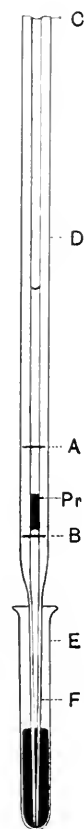


Fig. 2.

two clear days at a temperature sufficiently low to inhibit bacterial growth, the precipitum will have packed sufficiently to be measured in

that portion of the tube which is calibrated between the marks *A* and *B*. (See Fig. 2, Pr., also represented in black in Fig. 1.)¹

After the calibrated tubes have been used the contents are washed out forcibly under the tap, a short piece of rubber tubing connecting the end *C* with the tap. When not in use the tubes, at any rate in cold weather, can be kept in running water, being rinsed out before use with distilled water and drained.

Apparatus for measuring the amount of precipitum deposited in the calibrated tubes (Fig. 1, at 2).

This apparatus is shown in the accompanying figure, placed in front of the tube-rack (1). The instrument consists of an iron stand *A* provided with a vertical iron rod *B* grooved proximally so as to prevent the rotation of the upper part of the apparatus, this slides up and down along the rod and is clamped at any desired height by the screw *C*. Screw *C* is rounded and protrudes into the groove in rod *B*. The horizontal piece of metal which is traversed by screw *C* carries two vertical tubes provided with racks and pinions at *D* and *D'* by which inner tubes *E* and *E'* can be raised or lowered independently of one another, a play of 5 cm. being allowed. Tube *E* carries a steel 10 centimetre scale *F*, which is held in a vertical position. The scale is graduated in .5 mm.

Tube *E'* carries a vertically attached quadrangular plate through which a tube *G* passes centrally at right angles. An indicator is attached to the quadrangular plate on a line with the centre of the tube *G*. Two tubes slide back and forth in tube *G*. The distal one, nearest the tube-rack, is closed by a diaphragm (shown separately at 3) provided with a quadrangular aperture having a width corresponding to that of the glass tubes containing the precipitum about to be measured; a fine blackened needle protrudes horizontally into the centre of this aperture in the diaphragm. The second tube *I*, sliding within tube *G* proximally, is open at its proximal end, but provided distally with a diaphragm having a fine horizontal slit traversing the centre. The inner parts of the tubes etc. are blackened.

By means of this apparatus the volume of precipitum within the glass tubes contained in the rack can be rapidly measured. The

¹ When, as sometimes happens, the precipitum does not settle entirely to the bottom, but becomes lodged in part in the upper portion of the tube, it can be made to sink by introducing a horse-hair which is twirled about and then withdrawn.

footboard of the rack (1) is placed in contact and parallel with the base *A* of the apparatus. The height of the apparatus is roughly adjusted by loosening the screw *C* and raising or lowering the upper part on the rod *B*, tightening the screw *C* again when the desired height is reached. The tube bearing the needle-carrying diaphragm is brought as near as convenient to the calibrated tubes without touching them. By racking at *D'* and looking through tube *I* the needle point is brought in position with regard to the lower meniscus of the fluid and precipitum in the glass tube. By racking at *D* the scale *F* is brought into suitable position with regard to the indicator, say at a centimetre graduation. Releasing screw *D* and racking again at *D'* in an upward direction the upper level of the precipitum is soon reached, and the indicator shows the distance travelled upon the scale *F*, the reading being facilitated by means of the small magnifier *H* which is carried at a suitable distance from the scale upon a rod attached to the quadrangular plate above described.

The Tube Rack (Fig. 1 at 1).

To facilitate the measurement of precipita contained in a series of tubes, we have devised a rack such as is illustrated in the accompanying Figure 1 (1). The rack is constructed of a convenient height with regard to the measuring apparatus (2), so that the calibrated portion of the glass tubes contained in the rack shall be on a level with eye-piece *I* when the apparatus is in a medium position. The rack is intended to fix the tubes in a vertical position, as near as possible to the diaphragm which carries the needle in the apparatus (3). The small test-tubes *J*, containing mercury, rest in conical depressions bored near one margin of board *K*, corresponding to numbered vertical grooves in board *L* above. The calibrated tubes rest on the bottom of the mercury tubes and fix the latter in position when held in the grooves above. These grooves are triangular in cross-section so as to hold the glass tubes correctly in position when pressed into the grooves by means of the horizontal rod *M*. The rod runs flush with the top of board *L*, and is held in place by a light spring at either end. The rod *M* feathers easily, and holds a series of tubes neatly in position. The tubes should be inserted between the groove and rod from below upward. A strip of blackened cardboard, resting on two light metal supports attached to the sides of the rack, serves as a background which facilitates the measuring of the precipita in the calibrated tube.

The base board of the rack is flush with the sides, and, being straight, serves as a guide when sliding the rack across the line of vision (through the apparatus 2). This sliding can be rendered accurate and easy by placing the rack upon an oblong glass plate of suitable length the sides of which are provided with guides. Beginning with tube 1 the readings are made one after the other by sliding the rack in one direction across the track of the apparatus.

The apparatus, which may be useful for other purposes, was made for us by Messrs W. G. Pye & Co., Granta Works, Cambridge.

A METHOD OF ESTIMATING FUTURE POPULATIONS.

By T. H. C. STEVENSON, M.D. (LOND.), D.P.H. (CAMB.).

It will perhaps be generally admitted that few of the practical problems which have from time to time to be faced by the Medical Officer of Health in his capacity of statistician present more difficulty than is often involved by the estimation of populations. This problem may be said naturally to present itself in three different forms, according as the estimate is required for a past, a present, or a future date. Practically, however, there are but two primary divisions of the problem, the one relating to dates since which a census has been taken (and its results published), and the other to dates subsequent to that of the last published census. This distinction is pointed out by Dr Cressy Wilbur in the "Thirty-second Annual Report (for 1898) on the Vital Statistics of the State of Michigan⁽³⁾." He there refers to the first class of cases as estimates of inter-censal, and the second as estimates of post-censal populations.

Inter-censal estimates are the subject of a recent paper by Dr J. Spottiswoode Cameron⁽¹⁾. He compares the results for Leeds obtained by a method of interpolation with those to be got by assuming the population to have increased in geometrical progression between the years 1891 and 1901. The discrepancies are not serious, and for most purposes the simpler method of geometrical progression may be accepted as yielding sufficiently correct results.

In the case of post-censal estimations, however, the difficulties are in all cases much greater, and vary in degree with the interval between the date of the last available census and that for which the estimate is required. Three subdivisions of this class of estimates may be made, according as the estimate is required for (a) a past or present date, (b) a date in the near future, or (c) a date in the more or less remote future. A sharp line of division is drawn between groups (a) and (b)

by the fact that for group (*a*) various auxiliary methods of checking estimates of population, such as those dependent on the number of inhabited houses and on the birth-rate, can be used, which are not available for group (*b*). The methods to be used for groups (*b*) and (*c*) must also differ, but there is in this case no natural boundary-line defining the point at which the distinction must be drawn.

The method of most general application, in this country at least, to groups (*a*) and (*b*) is that of the Registrar-General, depending on the assumption of the continuance of the last ascertained *rate* of increase or decrease, and, consequently, on a future increase or decrease in geometrical progression.

Where records of migration are kept, estimates in group (*a*) are best made by adding to the natural increase the excess of immigrants over emigrants, or *vice versâ*. Thus in 1902 the population of New Zealand increased by 20,272, made up of excess of births over deaths 12,280, and excess of arrivals over departures, 7992. This method, however, can never be of general application, owing to the impossibility of tracing and recording the internal migration of any country. The method of assuming increase in arithmetical instead of geometrical progression is preferred for Michigan by Dr Wilbur in the paper referred to, and is shown to give results more closely approximating to the ascertained facts than those got by assuming increase in geometrical progression.

The Registrar-General himself, in the compilation of his published estimates, has occasion to use his method only for cases in group (*a*). But as it is used unchecked by the various other methods available for this group of estimates, the distinction in the case of his returns between groups (*a*) and (*b*) may be said to disappear. The method of estimating a population for the year 1908 which would be used in 1904, the case then coming under group (*b*), is the same as that which will be used for the returns in 1908, when the case will come under group (*a*). The circumstances of the case render it unnecessary for this method to be used in these returns for any date more than ten years subsequent to that of the last preceding census, consequently the example of the Registrar-General cannot be appealed to as authorising its use for any more distant date.

Even with this limitation the method at times yields very misleading results. Thus at the census of 1891 the population of Salford was found to be 20·9 per cent. below the estimate based on the rate of increase in 1871—81⁽²⁾. The period within which the method may be used with some degree of confidence will vary with the circumstances of

the particular locality, but the facts above referred to seem to suggest that there is no ground for assuming the applicability of the method for any date more than ten years subsequent to that of the last preceding census. Its use for more distant dates can easily be shown to be in many cases quite out of the question. If, as is not unfrequently the case, an estimate is required of what the population of a suburban district adjacent to a large town is likely to amount to in 20 or 30 years, this method will frequently furnish results which are absurdly high. When such districts are first opened out for building purposes the intercensal rate of increase may be very high, as much as 400 per cent. in some instances. In such cases the continuance of an equal rate for even 20 years longer might result in a population denser than that of the central part of the city, to the overflow of which the filling up of the outlying district is due. Obviously a method cannot be relied upon which is apt, in a comparatively short time, to make the suburbs more densely populated than the centre of the city, or perhaps than the maximum to which density of population ever attains in this country.

It must be recognised then that, while the accepted methods give more or less satisfactory results for estimates relating to the past, the present, or the immediate future, the only one of them which from its nature is applicable to the future at all cannot be relied upon for an estimate relating to a period more than a few years ahead. If in view of this we seek for further guidance than the last ascertained rate of increase, we find that as it was the density of population obtained by this method which demonstrated its inapplicability, so in devising a better method the influence of increasing density ought if possible to be taken into account.

Doubtless other circumstances than the density of population will modify the rate of increase, such as trade conditions, transit facilities, etc., influencing migration mainly, and alterations in the birth and death rates, influencing the natural rate of increase. Although we may probably look forward to a continuance, for some time longer at least, of the present steady fall in birth and death rates, still as the changes occurring tend to neutralise each other in their effect upon natural increase of population, it seems best not to attempt any correction for anticipated alterations in these rates. The conditions influencing migration twenty years hence, apart from density of population, cannot be anticipated at the present time, so alterations in these factors cannot be allowed for; in other words we are bound to assume the continuance of past conditions because we cannot in any degree foresee their future

alterations. The one condition of which the change can to a certain extent be anticipated and allowed for is density of population. Consequently the method to be described assumes, *faute de mieux*, the continuance in all other respects of past conditions, but modifies the results obtained in order to allow for the influence of increasing density of population.

In view of the very misleading results obtainable by the application of the method of geometrical progression in such cases Dr Thresh, Medical Officer of Health of the county of Essex, who required an estimate of the probable population in 1921 and 1931 of certain districts in this county bordering upon the county of London and the county borough of West Ham, suggested that in framing it advantage might be taken of the past experience of West Ham and East London. The present paper describes a method devised for the purpose of utilising this suggestion.

The districts of greater London to be estimated for were East Ham, Barking, Ilford, Leyton, Wanstead, and Walthamstow.

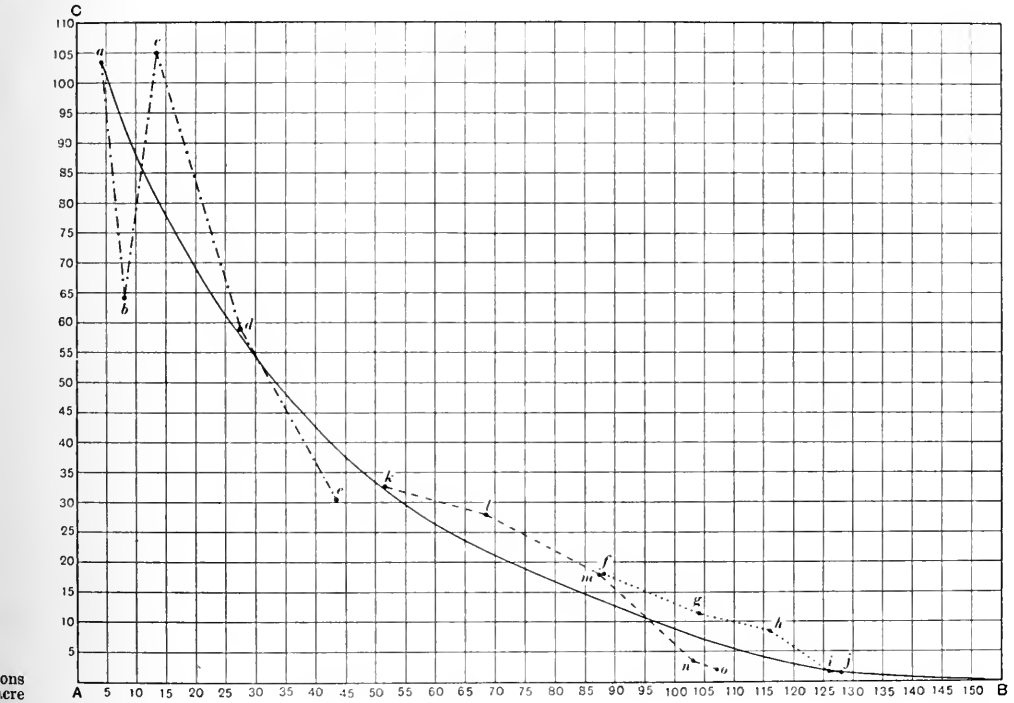
They have been filling up at an exceedingly rapid rate in the recent past, and in the case of some it would be absurd to anticipate the continuance till 1931 of an equal rate of increase. Taking the case of East Ham for instance, the population here was 32,713 in 1891 and 96,018 in 1901. The continuance for the next thirty years of an equal rate of increase would produce a population in 1931 of about 2,428,000, or 742 persons per acre, whereas the density of population in Whitechapel in 1901 was about 207 persons per acre.

As the districts in question are contiguous, and, on the whole, similar in character, it has been thought best to treat them as a whole instead of estimating separately for each, and they will therefore be referred to here for the sake of convenience, as the "combined districts."

The neighbouring borough of West Ham was selected to furnish guidance as to the probable course of their further development, as it may be said to have occupied thirty years ago the developmental position in which the "combined districts" find themselves to-day, and as the type of district is on the whole similar. As, however, the West Ham figures do not indicate the probable rate of increase beyond the point at which a density of 43·5 persons per acre has been attained, they have been supplemented by those for the "East End" Registration Districts of the county of London, of which, taken as a whole, the density in 1851 was 88, and in 1901 130 persons per acre. The

CURVE OF RELATIONSHIP OF INCREASE OF POPULATION
TO DENSITY IN EAST LONDON 1851—1901.

increase per cent. in
population during ten years



Figures, upon which curve is founded	a	= percentage increase (103.7) in West Ham population during	1851—61, the density in 1851 being 4 persons per acre.
	b	" " (64.1) " " "	1861—71 " 1861 " 8.1 " "
	c	" " (105.0) " " "	1871—81 " 1871 " 13.4 " "
	d	" " (58.9) " " "	1881—91 " 1881 " 27.4 " "
	e	" " (30.5) " " "	1891—1901 " 1891 " 43.5 " "
	f	" " (18.2) in East End Registration Districts	1851—61 " 1851 " 88 " "
	g	" " (11.5) " " "	1861—71 " 1861 " 104 " "
	h	" " (8.6) " " "	1871—81 " 1871 " 116 " "
	i	" " (1.6) " " "	1881—91 " 1881 " 126 " "
	j	" " (1.4) " " "	1891—1901 " 1891 " 128 " "
Figures, not used for construction of the curve, but applied after- wards as a test	k	" " (32.7) in (Bethnal Green, " " Mile End and " " Poplar	1851—61 " 1851 " 51.5 " "
	l	" " (28.1) " " "	1861—71 " 1861 " 68.4 " "
	m	" " (18.0) " " "	1871—81 " 1871 " 87.6 " "
	n	" " (3.7) " " "	1881—91 " 1881 " 103.3 " "
	o	" " (2.0) " " "	1891—1901 " 1891 " 107.2 " "

numbers of persons per acre in West Ham and in the East End Registration Districts at each census from 1851 onwards have been ascertained, as well as the corresponding percentage increase of population occurring in the ensuing decennium. Thus in 1881 the density of population in West Ham was 28·6 per acre, and the rate of increase in the following ten years was 58·9. The method adopted assumes that when the density of the "combined districts" amounts to 28·6, their rate of increase in the following ten years will also approximate to 58·9 per cent. Having obtained the rates of increase corresponding to ten individual density figures (five censuses, 1851—1891, and two communities being used) it was necessary to deduce from them the probable rate of increase corresponding to any given density figure.

The graphic method of interpolation was used for this purpose, and the curve of rates of increase obtained is represented in the figure, p. 211. The method of its construction is as follows: The different degrees of density of population, measured in persons per acre, from 0 to 150, are marked off along the abscissa line *AB*, and on the line *AC*, parallel to the ordinates, a scale of percentage decennial increases is marked. Then the different percentage increases of population recorded for West Ham and for the East End Registration Districts are charted vertically above the figure on the abscissa line *AB* representing the density of population recorded at the census marking the commencement of the ten yearly period during which the increase occurred. For instance, the West Ham increase of 58·9 per cent. during 1881—1891 is charted at the point marked *d* above the point on the line *AB* corresponding with the density of population of West Ham in 1881, namely 28·6. When this is done in each case it is found that, with one exception in the case of West Ham, the percentage rate of increase falls as the density increases. From the two sets of points thus determined, which are united by continuous black lines in the diagram, a curve has been drawn, filling in the gap between them (between densities 43·5 and 88) and smoothing off the irregularities met with, which, except in the instance referred to, relating to the early development of West Ham, are not very great. From this curve a rate of increase for the following ten years can be read off corresponding to any given density, and by means of it the estimate for the "combined districts" was prepared. Thus their density at the 1901 census was 14·9 persons per acre, and the rate of increase determined by the curve to correspond to this density is 78%. At this rate the 362,021 inhabitants of the "combined districts" would

become 644,397 in 1911. This is equivalent to a density of 26·6 per acre, and the corresponding increase as determined by the curve is 59%, which gives a population in 1921 of 1,024,591.

The simple curve obtained by following merely the general trend of the recorded figures is used rather than the irregular curve which would result from following the recorded experience in every case, because it seems likely that by doing so the influence of circumstances other than density of population is more or less eliminated. If the density were the only influence at work in determining the rate of increase we must suppose that it would act steadily, not irregularly, and that some such simple curve as is here constructed would represent its effect. If that is so then the irregularities of the series of recorded figures must be due to variations from time to time in local conditions other than density of population, and therefore are, for our purposes, best disregarded. But while it seems reasonable to suppose that a curve expressive solely of the influence of density would be simple, it by no means follows that, if it could be accurately ascertained, it would correspond with the curve in the figure. All that is claimed for the latter is that it represents an attempt to express the general trend of the recorded figures, and that no curve differing very widely from it would do so, or would be likely to represent the influence of density.

The estimates obtained from the curve, 1,024,591 for 1921 and 1,454,919 for 1931, may seem very high for districts containing in 1901 only 362,021 souls. London has however in recent years overflowed with great rapidity into these districts. Figures for East Ham have already been quoted. Its population was 10,706 in 1881, 96,018 in 1901, and is now estimated at about 115,000. Some of the other districts give figures almost as remarkable. If the method of a continuance in geometrical progression of the recent rate of increase is applied to the "combined districts" as a whole the estimate for 1921 becomes 1,558,869, an excess of over half a million over that obtained by the method adopted. This difference illustrates the extent to which an estimate based only on previous rate of increase is modified when the influence of increasing density is taken into account.

On the other hand, in the absence of a rational method of estimation, such as that founded on the basis of density is believed to be, the probable increase of population is liable also to be greatly underestimated. For instance, West Ham and other portions of the districts now dealt with have been said recently to be "full up," and not likely to increase in population. The truth of such a statement is best tested by

comparison with the experience of other similar districts, that is, on the density basis. When this is done we find that the density of West Ham in 1901 was 56·8, and that the three adjoining districts of Bethnal Green, Mile End, and Poplar had a combined density in 1861 of 68·4, with a percentage increase in the following decade of 28·1. Nor can it be said that conditions have altered so much for the better since that time that the analogy is inapplicable. The density of the East End Registration Districts was 126 in 1881, but an increase to 128 in 1891 and to 130 in 1901 occurred notwithstanding. Recent rehousing estimates for the London County Council have assumed a density of 200 per acre.

The above three have been selected from amongst the seven East End Registration Districts in order to test the curve already obtained as above described. Of the seven districts these three alone have grown to any extent since 1851, and so we may take it that the other four, which had a density of 168 persons per acre in 1851, and 175 in 1901, had almost reached the limit of density at the earlier date. The three selected, however, lying further from the City than the other four, had not reached nearly the same stage of development in 1851. Their density at that time was 51·5, and in 1901, 109·3. The East End Registration Districts therefore may be divided into an inner and an outer group, the history of the outer, as represented by the dotted lines in the figure, furnishing a test, to a certain extent, of the accuracy with which the graphic method has enabled the gap left between the records of West Ham, which cease at a density of 43·5, and those of the East End Registration Districts as a whole, which commence at 88, to be filled in. The increase in these three districts, following their density of 51·5 in 1851, namely 32·7, almost exactly corresponds with that read off the curve. The 1861—71 and 1871—81 increase rates are a little above, and the 1881—91 and 1891—1901 rates about the same amount below, those given by the curve for the corresponding densities. It follows that if these figures had been ascertained before the curve was drawn its form would not have been altered, as it continues to express the average of all the data secured.

The particular case dealt with is no doubt an especially favourable one for the application of the method described, for it has been possible to secure instances of neighbouring and approximately similar districts at all stages of development, but it is believed that the materials for its application exist in many other cases, though generally, perhaps, in a less complete form. When it is desired in any case to estimate the

probable increase of population in any outlying suburb there must often be some inner suburb whose past record can be used for the construction of such a curve as that described. When this can be done it is anticipated with confidence that the result will be more reliable than any to be obtained by the method of guess-work, however discreet and well-informed, or by assuming the maintenance of a past rate of increase, provided that the condition of similarity between the populations is borne in mind. It will be understood of course that only the third group (c) of post-censal estimates is being discussed, and that the method is not recommended for estimating populations of a few years hence. Nor does it seem likely to be useful for estimating the future populations of rural districts or of the central parts of large towns. These, however, are generally much more stationary than the suburban districts, and it is in the case of the latter, where the estimates are of greatest importance and greatest difficulty, that it is believed that substantial assistance will be afforded by the method described.

It will now be apparent why the method of arithmetical progression may well give better results, as tested by recorded facts, than that of geometrical. The latter method entirely ignores the influence of increasing density, and the curve, analogous to that in the figure, that must be traced if it be relied on, is represented by a horizontal straight line (the rate of increase remaining the same whatever be the density). The method of arithmetical progression on the other hand involves the assumption of a rate of increase decreasing as the population becomes more dense, and therefore, for communities whose space is limited, will more nearly approximate to the truth in the long run. The limitation of space is a necessary condition however. If we wish to estimate the future population of a borough which will from time to time enlarge its boundaries we shall probably best ascertain it, for the borough as enlarged, by geometrical progression. If, on the other hand, we wish to ascertain the future population of a definite restricted area, the use either of some such method as here described, or of the method of arithmetical progression, should generally give better results than geometrical progression. Similarly, if a country or a province is mainly agricultural the element of density comes in, since the amount of land available for crops or pasture is strictly limited, and, given free migration, the method of arithmetical progression would probably be preferable. But if the country or province is industrial, the space available being practically unlimited, the element of density does not

come in to any great extent, and as in the case of England, the method of geometrical progression may be expected to yield good results.

In conclusion, reference may be made to two obvious disadvantages of the "density method" described. The experience relied on is not that of the district itself but of some other, though similar, district, and the experience used may be many years old. These are both grave disadvantages, and a method involving them must be, at best, unsatisfactory. The nature of the problem, however, is such that a satisfactory solution is impossible, and the method described has seemed, in the particular instance given, to be less unsatisfactory than any other which suggested itself.

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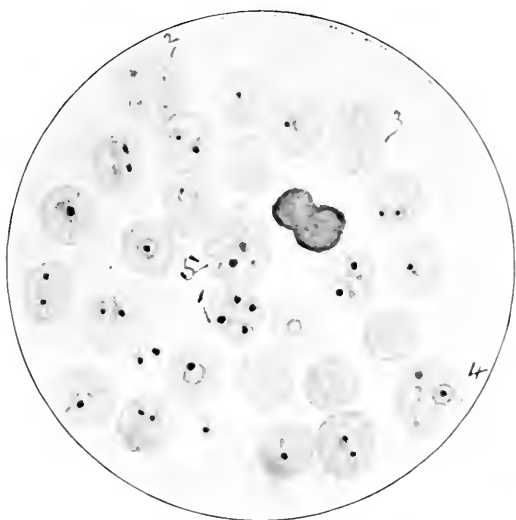


Fig. 1.



Fig. 2.

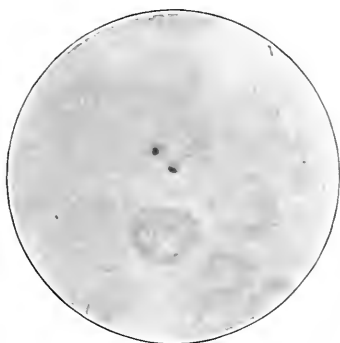


Fig. 3.

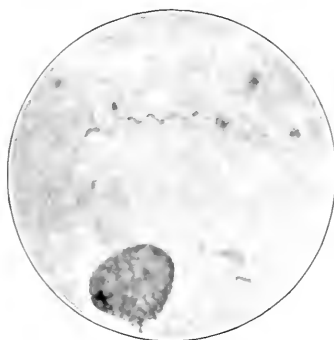


Fig. 4.

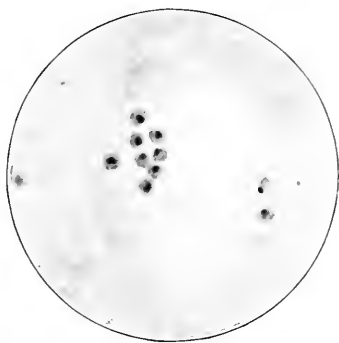


Fig. 5.

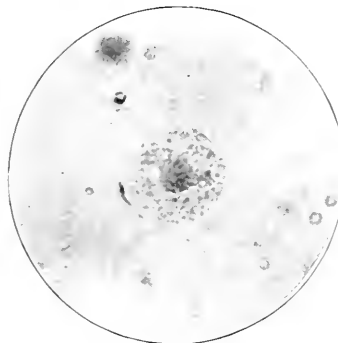


Fig. 6.



A CONTRIBUTION TO THE STUDY OF PIROPLASMOSIS
CANIS—MALIGNANT JAUNDICE OF THE DOG
(HUTCHEON).

By THOMAS BOWHILL, F.R.C.V.S.,

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ON visiting a farm near Grahamstown for the purpose of examining some cattle, our attention was drawn to a young dog which was sick. The owner stated that he had been unable to keep a dog on the place, that they all died.

An examination of the dog revealed the following symptoms: Temperature 104° Fahr.; biliary injection of the visible mucous membranes; animal semi-comatose and covered with ticks. Diagnosis: Malignant Malaria.

A little blood was taken from the animal's ear and films prepared, which we stained and examined on our return to Town. We found the red blood corpuscles teeming with the *Piroplasma canis*. The dog died the following morning. The cadaver was brought in and a post-mortem examination was made, and a number of films prepared from the kidneys, liver, blood, etc., in order to compare the various forms of the parasite present with those previously described by other investigators.

The examination of the literature at our disposal revealed the fact that various forms of the *Piroplasma canis* have been described by different writers, but we think the accompanying photograph (Plate XI, Fig. 1) of a drawing by one of us (Le Doux), also the photomicrographs of the parasites observed by us in the films, and especially those prepared from the kidney of this dog, show a few forms of this parasite which we believe have not hitherto been described in connection with Malignant Malaria of the dog.

The staining methods adopted were modifications of Romanowsky's original method, whereby we were able to stain the karyosome of the parasites a bright red and the remainder blue (see Fig. 1, also photomicrograph Fig. 5).

Several endoglobular parasites were observed which showed long flagella-like processes, some with two bulbs on the flagellum and some with only one at the end (see Fig. 1, 3 and 4, also photomicrograph Fig. 3). In some of the corpuscles parasites were present very similar to those found in human malaria but differing in not forming any pigment (see photograph Fig. 1, 5). Two pairs of parasites were observed in some corpuscles, the parasites being connected by a distinct flagellum-like process (staining blue), similar to those described by Lignières in *Piroplasma bovis* found in the blood of cattle suffering from "La Tristeza" in the Argentine Republic (see Fig. 1, 2, also photomicrograph Fig. 2).

Numerous free parasites were also present in the blood, and, in many corpuscles, "residual bodies" were observed, almost identical with those of human malarial infection. As many as eleven parasites were counted in one corpuscle. In a few instances we thought we observed flagellate bodies entering infected corpuscles. The flagellate bodies are illustrated in photomicrograph Fig. 4. The photomicrographs ($\times 1000$) which accompany this article were taken by one of us (Bowhill) from the same slides as those used for the drawing of which Fig. 1 is a photograph.

A number of ticks were found on this dog, most of them being the common blue tick *Rhipicephalus decoloratus*, and *Haemophysalis leachi* proved by the Government Entomologist, Mr Lounsbury, to transmit the parasite of this canine malady.

One of us (Bowhill) observed flagellate forms of *Piroplasma bovis* (Rhodesian fever of cattle) such as are represented in photomicrograph Fig. 6 ($\times 1000$; taken in 1902). The parasite consists of an enlarged elongated portion running into a delicate undulating "flagellum" upon which, on close examination, may be seen two minute bulbous protuberances. The spherical body alongside the parasite is a leucocyte.



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.

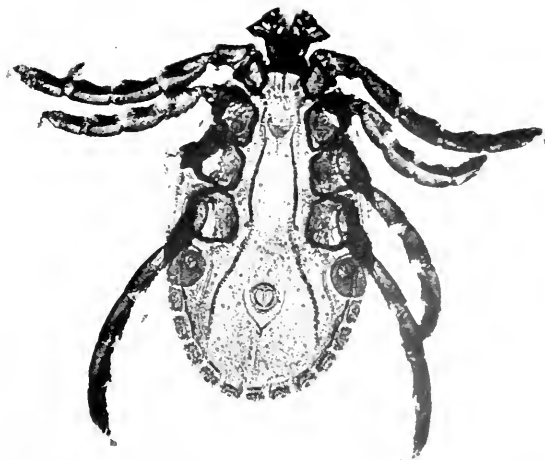


Fig. 5.



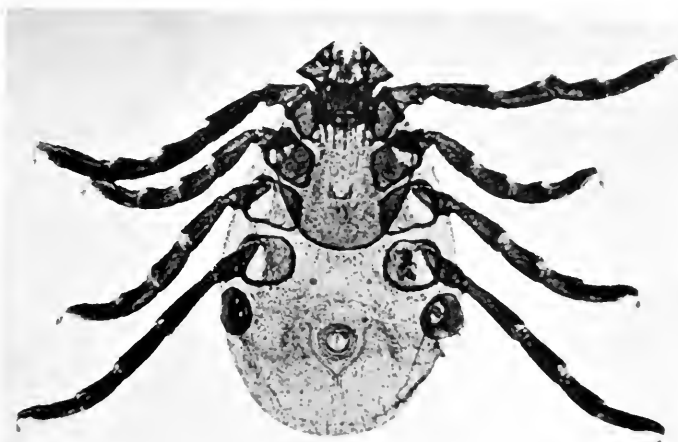


Fig. 6.



Fig. 7.



CANINE PIROPLASMOSIS.

I.

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1. Introduction.

THE disease which is the subject of this paper is one of a group of diseases affecting different animals, and caused by parasites possessing very similar characters. The diseases produced by parasites of the genus *Piroplasma* appear to closely resemble each other, and for this reason it is well to follow the terminology adopted by French investigators in speaking of any one of the diseases in question as a

Piroplasmosis. Not only are the parasites similar in these diseases, but they also appear, in all cases where the subject has been worked out, to be transmitted by species of Ixodoidea or Ticks.

All of the diseases in question possess considerable economic importance, for they are amongst the most devastating which affect domesticated animals in many parts of the world, and there is reason to believe that man may also be attacked by parasites of a similar nature. It is possible that the parasites affecting a given animal, although apparently similar, may belong to different species according to the species of tick which act as carriers in conveying the disease. Further investigations are however required to determine this.

Bovine Piroplasmosis is perhaps the most important of this group of diseases. It will suffice here to mention that it has been observed in America over a large area in the United States, in the West Indies (Porto Rico, St Thomas), in the Argentine Republic, Southern Venezuela, Uruguay, and apparently in Brazil. The disease has also caused vast monetary losses in Australia, and in Africa, where it has been observed in the North, South, East and West (Algiers, Egypt, Uganda, Cameroon, Cape Colony, German E. Africa, etc.). In Europe the disease occurs in S.W. Russia, Bulgaria, Hungary, Roumania, Turkey, Italy, Sardinia, France, Germany, Finland, Norway, and there is reason to believe in parts of Great Britain. In all cases the disease has been found associated with the presence of ticks on the affected cattle. The species *Rhipicephalus annulatus* (Say) was proved to transmit the disease by Smith and Kilborne and numerous observers since. This species occurs in America (localities mentioned above), also in many parts of Africa and Asia, and in Australia. *Ixodes redurivius* would appear to play a similar part in Europe, where it is widely distributed. This species is also suspected of transmitting the canine parasite (see p. 223). Judging from the results of inoculation experiments on animals other than bovine, the parasites encountered in cattle are species peculiar to cattle. Theiler and Koch however distinguish the parasites causing Redwater from those causing Rhodesian fever, not only on morphological grounds, but also because animals which are immune for the one disease are not immune for the other. It would appear as if Rhodesian fever of cattle may be transmitted by *Rhipicephalus shipleyi*, a species described in 1903 by Neumann; Mr Lounsbury has, however, just informed me in a letter (24. III. 1904) from Cape Town that *Rh. appendiculatus* carries this disease, and that "a single pathogenic specimen has sufficed for an infection." Lignières (1900) believed

that there were two species of *Piroplasma* causing similar diseases in cattle in the Argentine, and that bovine piroplasmosis in France (mal de brou) was due to still another parasite. It is impossible for me to enter further on the subject here, but what I have said will suffice to show for the present that when we speak of *Piroplasma bovis* we may be speaking of different species of parasites, and that it is well in all cases to state with what species of tick the parasite is associated and where the disease it causes is prevalent geographically, and even then it is not impossible that one species of tick may be able to carry two different species of parasites, given a suitable opportunity. We shall see below that *Ixodes reduvius* is suspected of transmitting the canine parasite in Europe.

Bovine piroplasmosis is known by many names: Texas Fever, Tick Fever, Blackwater, Redwater; Mal de brou, Malaria des bovidés, in France; Piscia sangue, Malaria bovina, in Italy; Weiderot, Rotnetze, Schwarzwasser, Maiseuche, Blutharnen, Waldkrankheit, etc., in Germany; Tristeza, Lomadera or Ringadera, in South America. All of which names suggest the prominent symptoms, which indeed very much resemble those of the canine malady especially considered in this paper.

Ovine Piroplasmosis was first observed in Roumania (Babès), subsequently in Italy, about Padua (Bonome), in Turkey, about Constantinople (Laveran and Nicolle), and in France (Leblanc and Savigné). Ziemann reported the existence of a similar affection in St Thomas, W. Indies, and Hutcheon and Robertson (1902) in S. Africa. Motas (1902—03) successfully infected four lambs through the agency of ticks (*Rhipicephalus bursa*) obtained from parent ticks fed on infected herds of sheep. He found these ticks in the larval stage to be incapable of communicating the disease. A perusal of Bonome's paper (1895) will show how very similar this disease, due to *Piroplasma ovis*, is to the canine malady. Like the canine and bovine malady, it is transmissible by inoculation with blood containing the parasites, and the disease occurs both in a mild and in a very fatal acute form (*see* Postscript).

Equine Piroplasmosis was observed by Bordet and Danysz; Theiler, Laveran, and others in South Africa; and in Germany by Ziemann, who moreover states that a similar disease prevails in Venezuela.

Human Piroplasmosis may prove to be the correct name for the disease called Rocky Mountain Fever, Spotted, or Tick Fever observed by Wilson and Chowning (1902), whose observations have received confirmation from Westbrook, Anderson, and others (1903). The disease

is probably transmitted by a tick (*Dermacentor reticulatus*¹), a species prevalent in the regions where the disease occurs, namely, in Montana, Idaho, Nevada, Wyoming, and Oregon in the United States. In all cases the attack was preceded by a history of tick-bites having been inflicted about a week previously. The disease only occurs in the spring and early summer, when ticks are active. The symptoms differ markedly from those observed in the bovine, ovine, equine, and canine maladies. Nevertheless the authors named describe and figure piroplasma-like parasites, and consider them to belong to the genus *Piroplasma* (personal communication; see Postscript).

There are other parasites which have been found in man by Leishman and since described by Donovan, Ross, Manson and Low, which Laveran named *Piroplasma donovani*. After seeing preparations of the parasite I quite agree with Ross and Manson in not considering them to belong to this genus. Ross has very appropriately named them *Leishmania* after their discoverer².

It seems in place here to refer to the pathogenic effects following the bites of different species of ticks in man and animals. Those interested can consult Nuttall (1899, pp. 43—49)³, some additional data are given by Sambon (1900)⁴. Particularly interesting are the recent investigations of Marchoux and Salimbeni (1903)⁵, which have demonstrated the part played by a species of tick (*Argas miniatus*) in the spread of a very fatal disease of the domestic fowl in Brazil. This disease is due to a protozoan parasite, a *Spirochaete*, similar to others found in man, in the goose (Sakharoff), and more recently by Laveran (1903)⁶ in cattle. The *Spirochaete* which causes the fowl disease evidently undergoes some development as yet unknown within the above-mentioned species of tick possibly after the manner of other

¹ Suspected in France of conveying *Piroplasma canis*, see p. 240.

² Leishman, W. B. (30. iv. 1903); *Brit. Med. Journ.* i. pp. 1252—1254; (21. xi. 1903) *Ibid.*; (6. ii. 1904) *Ibid.* i. p. 303. Donovan (28. xi. 1903), *Ibid.* ii. p. 1401. Ross, *Ibid.* p. 1401; (1904) *Ibid.* i. p. 160. Manson and Low (1904), *Ibid.* i. p. 183. Laveran (3. xi. 1903), *Bull. de l'Acad. de Méd.*; (7. xii. 1903) *Comptes Rendus de l'Acad. d. Sc.*

³ Nuttall, G. H. F. (1899), "On the rôle of insects, arachnids and myriapods, as carriers in the spread of bacterial and parasitic diseases of man and animals." A critical and historical study. *Johns Hopkins Hospital Reports*, viii. 154 pages, 3 plates. Published also in part in *Hygienische Rundschau*, 1899, Bd. ix. See especially pp. 402—408.

⁴ Sambon, L. W. (1900), "Ticks and Tick Fevers," *Journ. of Tropical Med.* ii. pp. 217—223.

⁵ Marchoux, E., and Salimbeni, A. (1903), "La spirillose des poules," *Annales de l'Institut Pasteur*, t. xvii. pp. 569—580.

⁶ Laveran (1903), "Sur la spirillose des bovidés," *Comptes Rendus*, p. 939.

protozoal parasites, such as those of malaria within the bodies of certain mosquitoes. It will be interesting to see if the *Spirochaete* recently found in cattle is also conveyed by a tick. In any case the work of Marchoux and Salimbeni is of fundamental importance, for it indicates in what direction research must be pushed to discover the mode of spread of relapsing fever in man, the parasite of which (*S. obermeieri*) has so long been known. Finally there are the investigations on "Heartwater" of goats and sheep in South Africa, a disease in which a tick (*Amblyomma hebraeum* Koch) is also regarded as the carrier of infection. I shall report upon this subject later.

Name of the Disease: Canine Piroplasmosis.

Previous to 1899 Hutcheon described the disease in South Africa as "malignant jaundice or bilious fever of the dog," stating that it was the most fatal disease affecting dogs in Cape Colony. It has also been called malignant malaria, hondziekte, malarial fever, and was formerly mistaken for distemper. In France it has been called infectious Icterus. All of these names more or less indicate prominent symptoms, but strictly speaking are unscientific. The jaundice is frequently absent, the disease may run a mild course, there may be but slight fever. I therefore prefer to name the disease after the parasite or parasites which cause it, namely Piroplasmosis, in accordance with the terminology adopted by French authors.

2. History, Geographical Distribution and Seasonal Incidence.

In considering canine piroplasmosis it will be well for the present to distinguish between the observations made in Europe and in Africa for the reason that the parasites causing piroplasmosis may belong to different species in different localities.

I. Canine Piroplasmosis in Europe.

Italy: The first to describe and figure the parasite were Piana and Galli-Valerio (1895). These authors, working in Milan, examined the blood of a dog suffering from fever, weakness, and slight jaundice after having hunted in marshy localities. They found the dog infested with ticks (*Ixodes redurivius* [L.]) and supposed that the latter might transmit the parasite whose resemblance to that of Texas fever was perfectly

evident to them. The parasites occupied 3—4% of the red blood corpuscles, and measured 3·5—2·5 μ , some parasites were encountered free in the plasma. The infected corpuscles contained 2—3—4—5 pyriform bodies. Some of the parasites showed amoeboid motion, this being excellently figured subsequently by Piana (1896) in a coloured plate, showing 24 sketches made of a parasite changing its form within 30 minutes, and protruding usually one or two pseudopodia from an ovoid or spherical body. Stained with thymol methylene blue, the parasites took a deep blue tint, the corpuscles being greenish. An unstained spot of round or oval form could be observed in the blue-stained protoplasm. The dog in question recovered—its blood showed leucocytosis, the erythrocytes were sometimes much enlarged, and nucleated corpuscles were encountered. In dogs previously examined, which had suffered from similar symptoms after hunting in certain localities, they noted at autopsy that the blood was less coagulable, that there was more or less icterus present, that the liver and spleen were congested. At times necrotic foci were observed upon the omentum near the pancreas. They noted the presence of haemoglobinuria, anorexia, prostration and emaciation, the fever recorded in one case reaching 40° C. It is perfectly obvious therefore that these authors were the first to observe the disease in Europe. They named the parasite *Pyrosoma bigeminum*, var. *canis*.

Celli (1900, p. 38) subsequently stated that similar parasites had been encountered in hunting dogs coming to the Campagna Romana from Lombardy.

In France the disease was first recorded by Leblanc (20. I. 1900) of Lyons. This author refers to "infectious icterus," being chiefly observed in hunting dogs. On examining a dog suffering from severe infection he found numerous haematozoa similar to those of cattle and sheep (*Piroplasma bovis* and *ovis*) in its blood corpuscles and free in the plasma. His communication on the subject was very brief, but clear and to the point, scarcely deserving the remark of Nocard and Motas that "la description qu'il fait du parasite est si brève et si peu précise qu'on ne saurait l'affirmer." Subsequently, after becoming aware of the investigations of Marchoux (mentioned below), Leblanc (17. II. 1900) reported having found similar parasites in four more dogs, the parasites according entirely with the description given by Marchoux.

Important contributions to our knowledge of the disease in France were those of Nocard and Almy (28. III. 1901) and Nocard and Motas (IV. 1902), in which a number of cases observed at the Alfort clinic

are described and much new matter presented which receives full consideration in the following pages. Five out of the seven cases occurring naturally in dogs had been already reported upon by Almy (10. x. 1901). Subsequently Nocard (1902) reported fifteen more cases of the disease in France, so we may conclude that the disease is fairly prevalent there. As Nocard (x. 1901) pointed out, the disease is quite different from the fatal icterus of young dogs, the cause of which is unknown.

II. *Canine Piroplasmosis in Africa.*

Canine piroplasmosis in *South Africa* was recognised as a distinct disease by Hutcheon as early as 1893 (p. 476). At that time he noted the destruction of blood corpuscles and the haemoglobinuria and dwelt upon the close resemblance between the canine disease and redwater of cattle. Cartwright-Reed (1893) reported upon a case of the disease at Herschel, Cape Colony, the diagnosis being arrived at by Hutcheon from the description given by the former. Hutcheon (30. xi. 1893) stated that the disease existed as a fatal epidemic affection in that region in the autumn and winter of 1893. Whilst Cartwright-Reed's note agrees fully with the classic descriptions of the disease, he noted that "the immediate cause of death was effusion of bloody serum into both cavities of the chest." As Hutcheon pointed out, the latter is not a constant phenomenon.

Both Hutcheon (1899, p. 399) and Robertson (1901, p. 331) state that Dr Carrington Purvis of the Bacteriological Institute, Grahamstown, was the first to discover *Piroplasma* in the blood of dogs affected with the disease. Robertson reported at the time that he had found the parasites in all cases of the affection. Shortly before this Koch (1898) appears to have seen similar parasites in *German East Africa*. In his report from Daressalam, he casually remarks that he had detected malaria-like parasites in one dog. He wrote (p. 107) that he had found a number of blood parasites "welche dem menschlichen Malaria-parasiten mehr oder weniger ähnlich sind, so bei vielen Arten von Vögeln, bei Reptilien, auch bei einem Hunde und namentlich bei Affen..." It is obviously this passage which is referred to by Marchoux (1900) and Nocard and Motas (1902) as evidence that Koch observed canine piroplasmosis "several times" in dogs in East Africa, but the original statement, above quoted, is altogether too vague to permit of any such conclusion. I however wrote recently to Professors Kolle and Kossel, at present in Berlin, on the subject, and they inform me that the

parasite Koch saw was *Piroplasma canis*. Possibly Martini (1903)¹ also refers to the above passage (he gives no reference) when he states that Koch observed this parasite in the Transvaal in 1897. Martini moreover states (again no reference) that Bitter of Cairo subsequently observed the parasite in dogs in *Egypt*, and that he figured the parasite. I have unfortunately been unable to trace this reference.

In *Senegal* the disease was observed by Marchoux (27. I. 1900), who found the parasites in 11 dogs which showed slight fever but no icterus. The parasites were most numerous during the febrile stage. The disease appears to have been benign. One of Almy's cases (No. 1, see p. 241) appears to have originated in *Tunis*.

The communicability of the disease by blood inoculation was first demonstrated, according to Hutcheon (1899), by Dr Carrington Purvis in South Africa, the observation being confirmed by Spreull in the same year (1900, p. 45). The communicability of the disease in France was demonstrated in a similar manner by Nocard and Motas (1902).

The transmission of the disease by means of infected ticks (*Haemophysalis leachi* [Audouin]) was first demonstrated by Lounsbury in South Africa. A similar demonstration is still wanting for the disease in Europe.

To justify the statement that the *Piroplasma* of Africa may be different from that of Europe I would refer to the geographical distribution of *Haemophysalis leachi*. This species appears to be almost confined to the African Continent. Neumann (1897, pp. 347—350) states that it was found in Egypt by Savigny, Mozambique by Karsch, Eastern Ethiopia by Antinori, West Africa at Pangolin by Mocqueryson, in Algeria by Fayet, in Sierra Leone by Trouessart, and Doumargue, in the French Congo, Transvaal, Cape Colony. Two males were however collected on *Felis tigris* in Sumatra. Almy (1901, p. 375) suspects *Dermacentor reticulatus* (Fabr.) of being the usual carrier of the parasite in France, for the reason that he found this species on dogs which developed the disease there. Salmon and Stiles (1900, p. 452) state that this tick is widely distributed, occurring in different European countries. Railliet (p. 713) records its presence in France and Italy, where it has been found on the ox, sheep, goat, and on man. Salmon and Stiles add that it has been found on horses, deer, roe-deer, fallow-deer, and the rhinoceros. Its presence on the dog does not seem to have been recorded before. This tick has been found in different parts of

¹ Martini (1903), *Archiv f. Schiff's- u. Tropenhygiene*, Bd. VII. p. 504.

the United States (California, Texas, New Mexico, Indian Territory, Tennessee, and Oklahoma). *Ixodes reduvius* [L.], on the other hand, appears to be widely distributed in Europe, and is a species which is suspected of being the carrier of the parasite in Italy (Piana and Galli-Valerio, 1895). This species is very common in France and Germany; it was found in Finland by Kossel and Weber, and has been found frequently in England. It is suspected of also being the carrier of *Piroplasma bovis* in these countries, where it attacks cattle, sheep, and a variety of animals. This species occurs moreover in North Africa, and in North America. As I have already indicated, these two species of ticks possibly transmit two species of canine parasites, but at present there is no proof one way or the other.

Where the disease occurs it appears to be confined to certain localities. Hutecheon (1893, p. 477) in South Africa, stated that it was very common in coast towns and districts, but comparatively rare in higher inland districts of Cape Colony. Nevertheless it prevailed about Herschel in 1893. In 1899 he reported (p. 398) that the disease was confined chiefly to the Eastern coast districts about 1884, but that in 1899 it was met with all over Cape Colony and the adjoining States, chiefly along the main lines of traffic. He first observed the disease at Port Elizabeth in 1885. Lounsbury (1901, p. 11) states that dogs at Cape Town are practically exempt from the disease, but that when such dogs are infected, this can usually be traced to their having previously been taken out upon the veld. Robertson (1902, p. 682) refers to Constantia as being "a notorious place for this affection of the dog."

Seasonal Incidence. According to Robertson (1902, p. 327) the disease prevails chiefly in summer and autumn at the Cape. In Italy cases were observed in April by Piana and Galli-Valerio (1895), in France in September and October by Almy. Further data are required regarding the seasonal prevalence of the disease both in Europe and Africa.

3. The Cause of the Disease.

The Parasite and the Tick which transmits it.

Our studies upon the parasite (*Piroplasma canis*) and the species of tick (*Haemophysalis leachi* [Andouin]) which transmits the parasite to dogs in South Africa are not yet completed, and I shall therefore defer

their detailed consideration, describing them in a future communication. For the present it will suffice to state that the parasite is a Protozoon, and that it occurs in the blood, throughout the body, being most numerous in the internal organs. Most of the parasites are encountered in the red blood corpuscles, but some, presumably derived from ruptured corpuscles in which they have multiplied, are encountered free in the plasma. The general appearance of the parasites is shown in the Plate accompanying the paper by Bowhill and Le Doux on p. 217.

The species of tick above mentioned certainly transmits the disease when infected with the parasite. To be infective the tick must be descended from a mother tick which has sucked the blood of an animal affected with the disease. After the parent tick has gorged herself with blood, she falls to the ground, and after a variable time lays a large number of eggs from which hexapod larval ticks issue in due course. The larval ticks attack a dog when they have an opportunity, fill themselves with blood, and, after about two days, drop off. According to the observations of Lounsbury, these infected larval ticks do not transmit the disease. After lying in the ground for a variable length of time the larval skin is shed and the eight-legged nymphal tick issues forth. Lounsbury has also been unable to show that the infected nymphal tick is infective. The nymph in turn attaches itself to a dog for a few days, and having gorged itself with blood drops to the ground. Here it undergoes its metamorphosis, as did the larva, and after this is accomplished, it casts its nymphal skin, and issues as an adult sexually mature eight-legged tick. It is the *adult* tick and *only* the adult which transmits the parasites, according to Lounsbury.

At Mr Lounsbury's request I have undertaken to control his observations with regard to the infective character of the ticks at various stages of their development. For the present, I can only say that I have successfully infected three dogs with infected adult ticks which Mr Lounsbury has kindly sent me. These experiments should prove convincing to any persons who may doubt the part played by ticks in similar diseases. The disease in question is, as far as I am able to ascertain, unknown in England, and it has been reproduced in England by means of infected ticks sent from South Africa. The control of the rest of Mr Lounsbury's most interesting observations will necessarily take some time, since weeks must elapse for the ticks to undergo metamorphosis from one stage to another. The fact that the tick harbours the parasite after having undergone two moultings subsequent to leaving the egg, and that the adult tick is capable of infecting after

having starved for five to six months, as I have found, certainly shows that the parasite must undergo some cycle of development, as yet undetermined, within the tick's body. It should dispose of the hypothesis advanced by Lignières (1900) in relation to the bovine parasite of a similar kind which causes "Tristeza" in the Argentine. Lignières considered it possible that the parasite might adhere to the external mouth-parts of the mother tick after sucking infected blood, and be deposited from the mouth-parts on the egg-surface during the act of oviposition, when, as others have observed, the mouth-parts play about the vulva. In proof of this possibility he cites experiments in which the injection into animals of crushed infected ticks and their eggs gave a negative result. Five years before Lignières, Hunt (1895) in Australia also reported the negative result of infection experiments by means of inoculations with the crushed bodies of young infected ticks which carried Texas fever. But surely such a crude experiment cannot be accepted as offering any evidence of the absence of the parasite from the internal organs of the tick! Even with the malarial parasites, mammalian and avian, whose cycle of development is known to take place in different species of mosquitoes, we do not know if a similar experiment would or would not yield a negative result. It is an experiment which I have suggested to several observers, but nobody has yet undertaken to carry it out.

The observations of Lounsbury to the effect that infected ticks may harbour, and not convey, the parasite until they reach the adult stage appears to have gained support from observations on ticks which convey similar parasites to sheep and cattle. Whereas *Rhipicephalus annulatus* as far as we know remains on cattle whilst undergoing its development from larva to adult, Kossel and Weber have found that *Ixodes reduvius* does not do so, the larva when gorged leaving its host. Furthermore, Motas (27. XII. 02, and 27. IV. 03), working with the similar disease of sheep (due to *P. ovis*), successfully conveyed it to four lambs by means of infected ticks (*Rhipicephalus bursa*). Here other conditions prevailed, the larva did not infect, and it stayed on the same spot on the animal's skin until it metamorphosed into a nymph. The gorged nymph dropped off, and became infective only on reaching the adult stage. It is evident, therefore, that ticks may behave differently in this respect. It is a matter of considerable practical importance with regard to the efficacy of practical measures of tick extermination, apart from the interest to science, to determine how different ticks behave in this regard. At present we know that some

ticks stay on throughout their different stages of development, others only during one moulting, whilst the species *H. leachi* drops off for each moulting.

These main points in the life-history of *H. leachi* are illustrated by the accompanying photomicrographs, for which I have to thank Mr Walter Mitchell, who took them from some specimens I have prepared. As already stated, I defer a more detailed description for the present.

EXPLANATION OF PLATES XII AND XIII.

THE TICK, *Haemophysalis leachi*.

Plate XII, Fig. 1. Blood-gorged female which has dropped from a dog and is beginning to lay eggs. The small legs are seen protruding from the distended body. When oviposition is completed the egg-mass may exceed the size of the original tick which lies beneath it half buried, finally the tick dies, having shrivelled like a raisin. Magnified about $2\frac{1}{2}$ times.

Fig. 2. Larva with six legs, as it appears after escaping from the egg. Legs curled under. $\times 20$.

Fig. 3. Nymph with eight legs, as it appears after escaping from the larval skin. $\times 20$.

Fig. 4. In a row down the centre are three gorged nymphs, which give rise to adult ticks, the adult male being shown on the left, the female on the right. The male has a more glossy back, owing to the whole dorsum being covered by the dorsal shield. This is not the case in the female, as will be seen by referring to Fig. 6 which follows. The adult tick appears about the same size as the gorged nymph from which it issues. $\times 2$.

Fig. 5. Mature male tick. $\times 20$.

Plate XIII, Fig. 6. Mature female tick, body somewhat broader than in male, dorsal shield extending but about half-way along dorsum. $\times 20$.

Fig. 7. Ventral view of capitulum of female, showing the hypostome with its rows of denticles lying centrally between the palpi. The denticles are directed backward, and together with the toothed mandibles situated dorsally (not seen in the photograph) serve to anchor the tick in the skin of its host. $\times 150$.

With the exception of Figs. 1, 2 and 3, the ticks have been prepared by maceration in caustic potash. The larvae in Fig. 2 are viewed dorsally, the rest ventrally, so that it is owing to the transparency of the chitinous structures that the dorsal shield is visible through the ventral wall in Fig. 6.

4. Incubation period.

In all cases a period of incubation of variable length precedes the febrile attack or onset of symptoms. This period can be most accurately determined under experimental conditions. Under natural conditions a dog showed symptoms 10 days after being on a tick veld (Robertson). In the cases reported from France by Almy, the period of incubation

appears to have been 10 days in two cases, apparently some weeks in one case, uncertain in others.

In three dogs which I successfully infected in Cambridge by means of ticks sent me by Mr Lounsbury, fever made its appearance on the 13th, 15th, and 16th days respectively, and Mr Lounsbury has reported a case in which a dog showed symptoms on the 21st day after the ticks were applied.

In blood inoculation experiments the period of incubation varies according to the mode of inoculation employed. After subcutaneous inoculation Robertson observed febrile symptoms on the 3rd day in one, and on the 4th and 5th day in two cases, and Lounsbury on the 7th day in a case which proved chronic, and on the 6th day in another. In France, Nocard and Motas observed febrile symptoms on the 5th and 6th day in two cases. I have observed corresponding periods of incubation, but in one of my dogs (Chart IV) it lasted 10 days.

As Spreull, and subsequently Robertson pointed out, intravenous inoculation shortens the period of incubation, thus in two of the latter's cases (Chart VI) the period was reduced to 3 and 4 days respectively. Nocard and Motas record two cases, one of which proved chronic, in which the period of incubation, following intravenous inoculation, lasted 3 days.

5. Symptoms.

The disease is ushered in by fever and loss of appetite, followed by increasing prostration, ending in complete helplessness. The breathing and pulse are accelerated, the former becomes laboured and finally shallow. Beginning with the onset of fever, there is a loss of weight which is greatly increased during the final stages, and appears to be more marked in long-continued cases. The mucous membranes soon become pallid and may show icterus. The skin may also become icteric. Haemoglobinuria occurs in a certain proportion of cases.

In the table which follows, I have recorded in separate columns the symptoms of canine piroplasmiasis as it occurs in the South African and French diseases. With regard to the former, I have incorporated the observations of Hutcheon, Robertson, and Lounsbury, and added some of my own. The data regarding the French disease are condensed and tabulated from the publications of Nocard, Almy, and Motas. Although the diseases appear practically the same, judging from the symptoms recorded in both columns, it appears to me premature to conclude that they are identical until the subject has been further worked out.

SYMPTOMS OF

AS OBSERVED IN

SOUTH AFRICA

Loss of appetite	Apparently a constant and early symptom. Observed in all the dogs I have experimented on. All food refused in later stages, may drink much water (but refuse milk, Hutcheon, 1899). This symptom also noted by Lounsbury and Robertson.
Vomiting	An early symptom in some cases (Hutcheon, 1899, p. 400), but not observed by me.
Prostration	Soon after the onset, in acute cases, the animal appears dull, listless, it lies down more than usual, later it does not change its position for hours. Toward the end it totters in its gait, finally it cannot stand, becomes completely helpless, and dies comatose. These appearances observed by me are also recorded by Hutcheon, Lounsbury and Robertson.
Breathing	Accelerated, subsequently laboured irregular, finally very shallow.
Pulse	Weak and rapid.
Mucous membranes and skin: icterus, anaemia	Visible mucous membranes become pale and icteric, may become chrome yellow in colour, and also the hairless portions of the skin (flanks, belly, prepuce and inner edges of ears). [Lounsbury and Robertson.] In 6 dogs infected by me in various ways (see Protocols) in Cambridge, icterus was very slight or not evident, but anaemia was well-marked in all cases. The tongue foul and furred, the teeth dirty, gums and mucous membrane of the mouth pale, bloodless, at times ulcerated (often icteric according to Hutcheon, who with Lounsbury and Robertson, noted foetid breath).
Body temperature	Fever recorded in all cases, and may be present when the dog appears well, thus constituting usually the first symptom. Fever starts at 104.2°—105.4° and oscillates or rises to 105—106.6°, even 107° F. In chronic cases (Chart V) there may be great oscillations in temperature which may fall below normal (97—98° F., about 36° C.) and again rise. Toward death the rectal temperature gradually falls far below normal, in three of my dogs 98.2°, 97.2°, 90° F. (32.2° C.) were recorded respectively when last taken.
Loss of weight	A marked loss of weight was recorded in four of my dogs, amounting: in Dog 3 (died in 4½ days) to 6.5%; in Dogs 5 and 7 (died in 8—9 days) to 14.3% and 17.7% respectively; in Dog 1 the loss of weight amounted to 12% during the last three days alone. The loss occurs chiefly toward the end. Emaciation appears to be more marked in chronic cases, see Chart V: Dog a "living skeleton."
Urine: haemoglobinuria	All the cases I have observed have been acute and haemoglobinuria was present, also albuminuria. The urine was claret or brownish-red in colour, or resembling coffee-grounds. Lounsbury and Robertson consider this brown colouring an unfavourable symptom, indicating a fatal termination. Haemoglobinuria was noted by Hutcheon (1899). It may be absent in fatal cases as in redwater (Robertson, p. 329). In one urine I examined (Mr Graham-Smith will report upon the other cases later) I found the reaction acid, albumen, haemoglobin, bile salts and pigments, a considerable deposit consisting of spermatozoa (chiefly), granular casts, epithelium, leucocytes, granular detritus, crystals of salts and a few erythrocytes. There were no spermatozoa or bile salts and pigment present on the day preceding death (Dog 1). No haemoglobinuria was observed in the chronic case recorded in Chart V.

CANINE PIROPLASMOSIS

THE DISEASE OCCURRING IN

FRANCE

Loss of appetite at onset in acute cases, and appetite not regained. Anorexia also observable in chronic cases.

A rare symptom, occurs in acute cases, intractable when present. Greenish mucus expelled.

In acute cases dog dejected, lies in a corner. Sensibility greatly lowered. Movements difficult, muscular twitching, especially of hind limbs, followed by paresis. Dog rises with difficulty and may tumble over. When temperature becomes subnormal dog ceases to move. Dies comatose. Only once were tetaniform convulsions seen to precede death (N. and M. p. 261).

In chronic cases lethargy and muscular weakness accompany the profound anaemia.

In acute cases respirations 36—48 a minute (accelerated), laboured, gasping, and at times, especially in young dogs, accompanied by whining sounds. Examination of thorax negative.

In acute cases beats 120—160 a minute, rapid, weak, thready, often intermittent.

In acute cases visible mucous membranes become pale, then cyanotic, and in some cases (inconstant) icteric. Icterus noted in the mucous membranes of the eye, mouth, and skin in 30 out of 63 cases observed (p. 260).

In chronic cases profound anaemia, rarely icterus. Mucous membranes very pale, skin dry, coat dull.

In acute cases fever at onset may exceed 40° C. (104° F.), is maintained usually 2—3 days, then the temperature falls below normal, even down to 33° C. (91·4° F.). Rarely temperature is seen to oscillate, then gradually fall. In young dogs which die very quickly initial fever may be absent, parasites appear in the blood and temperature sinks until death.

In chronic cases fever usually absent, slight when present, rarely exceeds 40° C. (104° F.). May be overlooked, lasts 36—48 hrs., then falls. In one case a "quartan fever," with remissions as in the human malaria observed.

Nocard and Motas do not mention anything about loss of weight in acute cases, but in chronic cases they state there is emaciation.

In acute cases: urine *albuminous* at onset before parasites can be found in the blood. Albuminuria persists until death, increasing with number of parasites present. *Haemoglobinuria*: urine pink, dark red, blackish, like prune-juice or coffee-grounds according to its degree. No erythrocytes in urine. Oxyhaemoglobin may amount to 2·5%. Haemoglobinuria appears soon after parasites are seen in the blood and in very acute cases persists until death and is found in bladder at autopsy. Haemoglobinuria inconstant, noted in 3 out of 6 cases by Nocard and Almy, this may be due to its being at times very transitory. Nocard and Motas observed more or less lasting and severe haemoglobinuria in 43 out of 63 dogs. *Bile pigment* present in cases showing icterus and haemoglobinuria. *Reaction acid*, only found neutral twice, alkaline once. Polyuria rare.

In chronic cases: urine usually *slightly albuminous* at start, condition lasting 15—20 days. *Haemoglobinuria* very rare, lasts 1—2 days. Urine may be icteric. *Reaction acid*, only once found neutral, this attributable to other causes (sugar found).

The Duration of the Disease, reckoned from the appearance of the first febrile symptoms until the occurrence of death, varies according to the acute, subacute, or chronic character of the disease. In extremely acute cases the illness may last only 24 hours, but it appears more often to last 3 to 6 days. In subacute cases the disease lasts about 10 days, and in chronic cases anywhere from 21 to 62 days. These statements are based upon the following table in which I have included all recorded observations possessing a suitable character in point of accuracy.

Disease acquired	Duration in days	Authority	Remarks
Naturally by Tick infection	{ 4 5—6	Lounsbury, Robertson Almy	1 case in S. Africa 1 case in France
Experimentally by Tick infection (<i>H. leachi</i>)	{ 1, 1, 5, 11 8, 9, 10½	Lounsbury, Robertson Nuttall	4 cases, two first in puppies 3 cases in Cambridge
Experimentally by subcutaneous inoculation with infected blood	5	Spreull	1 case
	2—3	Robertson	A general statement
	4½	Nuttall	1 case in Cambridge
	9, 10, 11	Nocard and Motas	A general statement, disease in France
	21—30—40, 60	„ „	Ditto, in chronic cases
	62	Lounsbury	Case recorded in Chart V, chronic
Experimentally by intravenous inoculation with infected blood	{ 3 4—5	Spreull Nocard and Motas	1 case in S. Africa A general statement

For convenience' sake I have included the observations by Nocard and Motas (1902, p. 273) upon the French disease in the above table. They say that the disease may last 36—40 hours in very young puppies, but that it usually lasts 3 days after the appearance of the parasites in acute cases.

I have not as yet had an opportunity of observing the chronic type of the disease, but through the courtesy of Mr Lounsbury I am able to present an unpublished record (Chart V) of a case lasting as long as 62 days from the onset of symptoms, and ending in death after what appear to have been unsuccessful attempts at recovery. At the end of about a month the dog had become a "living skeleton," subsequently its temperature fell to subnormal on three days and then again rose, pursuing an irregular course throughout. Toward the end of the second month the dog became blind in both eyes. Robertson (5. VI. 1902, p. 682) says that many dogs, after practically recovering from

the jaundice, "died of anæmia and prostration long after all clinical symptoms of the disease had disappeared." Hutcheon (1899) attributed death to exhaustion. Others have attributed death to collapse and weakness due to the drain on the blood consequent on the hæmoglobinuria, but Lounsbury points out the fact that deaths occur in the absence of hæmoglobinuria. It appears from the experience of Nocard and Motas (1902, p. 258) in France that the acute disease is usually fatal, whereas the chronic type usually ends in recovery. In such cases recovery proceeds very slowly, lasting 6—12 weeks.

6. Pathology.

To avoid confusion between the canine piroplasmosis observed in Europe, notably in France, and the South African disease, I have compiled the following brief table relating to the pathology in the same way as I did the one for the symptoms. Mr Graham-Smith has made the pathology of the disease the subject of special study, and will report thereon in due course. The appearances at autopsy are identical in naturally and experimentally infected dogs. In dogs dying from the acute type Nocard and Motas state that there may be an entire absence of abnormal pathological appearances at autopsy. According to these authors lesions are most marked in chronic cases, and the changes observed in the various organs point to their depending upon the "extreme dilatation of the capillary network by the accumulation therein of corpuscles mostly gorged with parasites¹."

Hæmatology.

The changes in the blood in the South African disease are being made the subject of study by Dr Wright, so that I shall leave it to him to report thereon in a later paper. On the other hand I herewith append an abstract of the observations recorded by Nocard and Motas (1902) for the French disease. What observations I have made agree very closely with those of the French observers. This might be expected from the great similarity between the descriptions of the symptoms and pathology, etc.

In acute cases of the French disease (p. 261) the blood appears profoundly altered, pale and watery. Coagulation is retarded, the coagulum softer and paler than usual, the serum tinted more or less

¹ Refer to p. 223 for the earlier observations of Piana and Galli-Valerio in Italy.

PATHOLOGY OF

AS OBSERVED IN

SOUTH AFRICA

External appearances	Great emaciation, extreme pallor of all visible mucous membranes and hairless portions of skin (Robertson).
Blood	Thin and watery (Lounsbury, Robertson). The serum tinged with haemoglobin.
Spleen	Much enlarged, congested (Hutcheon). Slightly enlarged, soft, pale, bloodless, like other organs scarcely stains paper when smeared thereon. Parasites therein numerous (Robertson). Little change observed in our cases.
Liver	Usually congested, at times inflamed, mahogany or saffron-colour. <i>Bile</i> dark green, usually thicker than normal (Hutcheon). Liver enormously enlarged, soft, "distended with watery fluid" (Robertson). But slightly changed macroscopically in our cases.
Kidneys	Congested more or less, at times oedematous, cortex dark brown (Hutcheon). Kidney pale and friable (Robertson).
Bladder	Normal, urine generally dark brown like "pontac" (Hutcheon). Urine may appear normal (Robertson). <i>See Symptoms</i> p. 232.
Serous cavities	Peritoneal and thoracic cavity may contain fluid (Hutcheon). Sometimes brownish serous exudation in thorax. In Lounsbury's chronic case (Chart V) pericarditis and pyothorax.
Stomach	Fundus generally inflamed, pyloric end normal, occasionally a few ulcer-like spots on rugae of stomach (Hutcheon).
Intestines	Catarrhal inflammation of small intestine, more intense about duodenum. Lumen contains viscid mucus often mixed with blood. Large intestine slightly but not uniformly inflamed, contains much viscid mucus (Hutcheon).
Heart	Pericardium contains variable amount of serous fluid. Ecchymoses around heart, largely on left ventricle (Hutcheon).
Lungs	Rarely affected, according to Hutcheon. I noted oedema and pinkish frothy fluid in the bronchi and trachea.
Bone-marrow	Congested.
Lymphatic glands	In one of my dogs the retroperitoneal glands appeared injected, the others unchanged.
Central nervous system }	Slight congestion in <i>some</i> of our cases.
Icterus	
	In some cases the tissues in general are yellowish (Hutcheon).

CANINE PIROPLASMOSIS.

THE DISEASE OCCURRING IN

FRANCE

Heart and large blood vessels contain soft clots composed mostly of fibrin, floating in haemoglobin-stained serum.

Often enlarged 3—4 × natural size, softened, dark, reddens on exposure to air. No changes may be noticeable in acute cases. Parasites numerous therein in either case. Splenic enlargement when present can be determined during life.

Usually congested, shows little change. Blood flowing on section is rich in parasites. *Bile* usually thick, syrupy, grumous or dark green, and distends gall-bladder.

In an acute case the size of liver was normal, colour yellowish, gall-bladder filled with syrupy bile.

Greatly congested usually, capsule easily stripped, revealing numerous petechiae. On section cortex seen to be congested, shows petechiae. Blood from kidney very rich in parasites. In an acute case yellowish-red fluid exuded on section.

Prune-juice-like urine found in an acute case.

Mucosa infiltrated and congested on a level with the duodenum in a few cases.

Pericardium contains yellow or bloody fluid. Not infrequently observe numerous petechiae about apex or beneath endocardium of left heart. Heart may be pale (acute case).

Often small apoplectiform foci. In young dogs dying quickly usually acute oedema and reddish foamy secretion in bronchi and trachea.

Usually intensely congested, foetal in appearance, soft, friable, rich in endoglobular parasites.

Rarely affected.

Nothing special, meninges slightly congested.

Intense icterus of mucous membranes, sclerae, skin and fat recorded in an acute case.

by haemoglobin. Where haemoglobinuria is succeeded by icterus, the serum appears deep yellow, showing a greenish reflex at times. Citrated blood, on standing, shows a layer of corpuscles corresponding to 1/5, 1/10, 1/15th of the plasma.

Whereas the erythrocyte count in the normal dog amounts to 6·5 to 7 million (Malassez method), a progressive decrease is observed in dogs affected with piroplasmosis. The decrease begins gradually with the onset of symptoms, and with the appearance of haemoglobinuria there is a sudden fall to 2 million or less. There is then a corresponding fall in the percentage of haemoglobin, namely to 13—12—6·4—3·5%. The erythrocytes are not simply reduced in numbers, they are altered, so that in stained films they appear of different sizes, some all the way from a third to two-thirds larger than normal, and taking the stain poorly. Nucleated erythrocytes are also observed especially in chronic cases. Erythrocytes containing many parasites are much enlarged, and, bursting, liberate the parasites. Parasite-containing corpuscles as well as parasites may be taken up by leucocytes (p. 271).

There may be considerable leucocytosis, the number of leucocytes being increased 2—3—4 times the normal, so that instead of having 7—8000 (normal) as many as 40,000 may be counted. The multiplication almost entirely affects the polynuclear elements, this being especially marked in slow running cases.

Nocard and Motas (p. 289) give the following record as typical for an acute case (see Chart VII, Case II).

Day	Erythrocyte count	Remarks
1	5,240,000	Intravenous inoculation with 2 c.c. of virulent blood
2	5,560,000	Dog well
3	5,960,000	Dog well apparently, temp. 40° C., few parasites
4	5,240,000	Dog dejected, pulse 124, many motile parasites
5 (morning)	2,600,000	Dog miserable, hind limbs paralyzed, temp. 35·6° C.
(afternoon)	2,200,000	Condition worse, temp. 33·5° C.
6	—	Found dead

In chronic cases (p. 264) the progressive anaemia is explained by the blood changes. Here the erythrocyte count falls to 2 or 1·2 million, a fall in numbers being especially noticeable after a fall of fever, and continuing after the parasites have apparently disappeared or are rare. A slow increase begins in 25—30 days in cases leading to recovery, a normal count being reached after 2—3 months. The loss of haemoglobin is less marked than in acute cases, thus, in a dog showing 2,760,000 erythrocytes the haemoglobin gave 9·5%. Stained films

show, even better than in acute cases, great differences in the size of the erythrocytes; they may be enlarged 2—3 times above the normal. The enlarged corpuscles take the stain feebly. Many nucleated corpuscles may be found, especially at the commencement of corpuscular decrease.

Leucocytosis is always marked, the count amounting to 15,000—30,000, and in one case even to 54,000. The increase of leucocytes is due equally to mononuclear and polynuclear elements. Where there has been fever, on the days succeeding a fall of temperature, leucocytes—mononuclears only—are encountered which contain erythrocytes laden with parasites, a condition rarely met with in acute cases. As recovery takes place, the erythrocytes increase, the leucocytes decrease, agglomerations of haematoblasts being however encountered.

The following record (p. 289), which I have also remodelled, is given as being typical of a chronic case, ending in recovery. The dog was inoculated intravenously with 2 c.c. of virulent blood obtained from one of Almy's naturally infected dogs (No. 3) (see Chart VII, Case I).

Day	Erythrocyte count	Remarks
1	5,840,000	—
2	—	Few parasites found
3	—	More parasites. Eating less
4	—	Dog dejected, appetite gone, numerous motile parasites
6	4,040,000	Few parasites found, lying down, mucous membranes pale
8	2,820,000	Profound anaemia, insensibility, very few parasites
10	1,520,000	Same condition, nucleated erythrocytes numerous, leucocytes = 54,000
15	1,200,000	Same condition, no parasites found, leucocytes = 10,000
18	2,120,000	Slight improvement, no parasites found
20	2,480,000	Distinct improvement, few nucleated reds, no parasites found
25	4,380,000	Improving rapidly, lively, hungry, mucosae pink, no parasites found
27	5,100,000	Haematoblasts numerous, but one parasite found
—	—	Dog considered cured

According to Nocard and Motas (pp. 277—278) it is especially in such cases as the preceding that phagocytosis is observed, that is in cases leading to recovery. The mononuclears may include as many as 2—6 infected corpuscles, which are more or less degenerated. Phagocytosis is observed in the peripheral blood, but is most marked however in the spleen, where it may be seen even in dogs dying of the acute disease. When the corpuscles included are numerous, the gorged leucocyte may simulate the cross-section of a capillary.

7. Natural Mode of Infection.

The part played by adult ticks, of the species *Haemophysalis leachi* (Audouin), in the transmission of the disease to healthy dogs has been mentioned in part on p. 226. A similar part has not been demonstrated for the European dog tick, but there is circumstantial evidence that it is capable of performing a similar rôle.

The following case, cited both by Lounsbury and Robertson (1902, p. 333), evidently serves as a type of the usual way in which the disease is acquired in South Africa: A small, aged pointer dog, belonging to a gentleman living in the suburbs of Cape Town, was taken out on the veld after having been kept at home for about two years. The owner stated that the dog had had the disease previously. Ten days before the last illness he took the dog shooting to Kuils, a very bad tick veld. The animal returned with ticks upon him, fell ill after a week, and died on the fourth day of illness.

There is no evidence pointing to any other mode of infection in nature. According to Robertson (1902, p. 327) the disease is not communicable by ingestion, nor when healthy dogs cohabit with diseased ones. As Lounsbury (1901, p. 10) points out, fleas (*Pulex serraticeps*) and dog-lice are evidently not suitable hosts for the parasites, for the disease has not been observed amongst dogs in places where these fleas abounded, and in which healthy and diseased dogs were housed together. The disease is moreover rare in suckling pups, although they suffer as much as adult dogs from fleas and lice.

As stated, there is no direct evidence to prove the agency of ticks as carriers in the disease observed in France by Nocard and Motas. Nevertheless they bring forward strong presumptive evidence. In all the cases recorded both by them (1902, pp. 274—275) and by Almy (10. x. 1901, p. 375), some 7 in all, the dogs which had acquired the disease under natural conditions had been recently covered by ticks, in most instances after hunting, about Alfort, in wooded or shrubby country, or being housed in tick-infested kennels. These authors suspect a certain species of tick, *Dermacentor reticulatus*, of probably being the usual carrier of the disease in France. Experiments carried out by Nocard and Motas with a view, if possible, of demonstrating this, gave negative results. This was doubtless due to their experimenting with the *larvae* of this tick, a stage which appears to be incapable of being infective, certainly in the case of *H. leachi*. The larvae of *D.*

reticulatus were observed to fall off soon after they were put on the dogs, and they were lost.

The histories of 5 cases of Piroplasmosis occurring in dogs brought to the clinic at Alfort between 2 Sept. and 8 Oct. 1901, recorded by Almy (10. x. 1901, pp. 375—379), are herewith appended in evidence of the part probably played by *Dermacentor reticulatus* in the transmission of Piroplasmosis in France:

Dog 1. Had been in Tunis¹, where it had fallen ill some weeks before, having been attacked by ticks there (species undetermined). When examined the dog was profoundly anaemic. Erythrocytes numbered 1,260,000. Piroplasma found. Dog died.

Dog 2. Had hunted in the department d'Eure-et-Loire, from 1 to 3 Sept. Attacked by ticks. Fell ill, 9 to 10 Sept., haemoglobinuria noted 11th and Piroplasma found in the blood. Icterus followed, and death on the 15th. Typical appearances at autopsy.

Dog 3. Had hunted two days before and numerous ticks were found on him. Came to Alfort 24 Sept., anaemic, erythrocyte count 2,500,000, Piroplasma found. 7 Oct. the erythrocytes numbered 4,100,000 and the dog was apparently recovering.

Dog 4. Received in the Alfort clinic 5 Oct., suffering from haemoglobinuria. Had been in the woods 10 days before, and been infected by many ticks. Piroplasma numerous. Icterus followed. 12 to 15 Oct. appeared to improve; 26th, temperature sank to 34° C., 27th, to 22° C. (per rectum), died during the night.

Dog 5. Admitted 8 Oct. to Alfort clinic, being off his feed. Many ticks observed on him during the previous days. Piroplasma found. Left clinic 24 Oct. apparently quite recovered.

It is evident from the foregoing, as well as from the observations in South Africa, that hunting dogs are chiefly affected, for the reason that they are more exposed to the attacks of ticks. It seems futile therefore to mention the objections advanced by Leblanc (10. x. 1901, p. 380), who claimed that the part played by ticks could not be important for the reason that dogs are frequently tick-covered without developing the disease. Leblanc's objections were fully met by Nocard (10. x. 1901, p. 381) when he stated that it was fallacious to conclude that all dogs must get piroplasmosis if attacked by ticks. To begin with, dogs are attacked by different species of ticks, some of which may not be suitable hosts for the parasite, and a suitable species of tick may frequently not be infected with the parasite. I would add that Leblanc's objection is of the same nature as that brought forward by certain persons against the part played by *Anopheles* in the transmission of malaria, and is based simply on ignorance of facts.

¹ May have been infected there.

8. Infection Experiments.

1. *Infection of dogs by blood inoculation.*

The first to communicate the disease from diseased to healthy dogs by means of blood containing parasites were Purvis and Spreull, in S. Africa. Hutcheon (1899, p. 399), who reported Spreull's observations, states that the injection of small quantities of infected blood caused the death of two dogs. The dog which received a subcutaneous injection of virulent blood showed fever on the 6th day and died on the 11th day after inoculation. The dog receiving an intravenous injection showed fever on the 4th day and died on the 7th day after inoculation. The more rapidly fatal disease following upon intravenous inoculation has also been observed by Nocard and Motas in the French disease. These observers (1902, pp. 263, 273) inoculated dogs subcutaneously, and intravenously and also into the muscles. Intravenous injection produced a more rapid and certain infection. Similar observations have been made by Robertson (1901, p. 331) and Lounsbury (1901, p. 9¹) at the Cape. Death followed 11—13 days after subcutaneous injection in their animals, the illness lasting 2—3 days. The observations made both in France and in South Africa show that the experimentally produced disease is in all respects similar to that naturally acquired. (Robertson, p. 329, Nocard and Almy (28. III. 1901).) I have considered the acute and chronic types of the disease on pp. 232—235. Also in Charts I—VII.

Robertson (1901, p. 331) transmitted the disease by inoculation through a series of 13 dogs and thought (p. 332) that the disease "was aggravated and rendered more virulent, by passage through dogs."

The amount of virulent blood required to produce infection appears to vary. In young dogs, which are more susceptible, Nocard and Motas (iv. 1902, p. 273) found the intravenous injection of 1 drop to be sufficient to kill, whilst adult dogs developed the disease after the

¹ Lounsbury (1901, p. 9) records two experiments made in conjunction with Robertson, in which death took place sooner in a dog inoculated subcutaneously. This is evidently due to this dog being young, and consequently more susceptible (see p. 245). The experiments were as follows:

- I. Irish terrier pup, 3 weeks old, received 3 c.c. of infected blood subcutaneously (9. ix. 1901). Parasites appeared in its blood after 6 days, death occurred on the 8th day.
- II. Adult dog, received 3 c.c. of infected blood into the jugular vein. Parasites appeared in its blood on the 4th, and the dog died on the 11th day after inoculation.

injection of 1 c.c. of virulent blood. Robertson (vi. 1902, p. 682) in Africa found the duration of the disease, ending in death, to vary "in certain cases with the quantity of infecting matter used." In the experiments reported by Lounsbury (1901), successful infection followed the injection of 2—3 c.c. of virulent blood.

2. *Infection of dogs by means of Ticks (Haemophysalis leachi*
[Audouin]).

The first experiments on the infection of dogs with ticks were made in South Africa by Lounsbury. The initial experiment, recorded by Robertson (1901, p. 333), was unintentional in so far as it was conducted with the view of studying the life-history of the tick.

A small rough-haired mongrel dog was kept for two months in close confinement in a glass case by Lounsbury, the dog serving as a host for ticks obtained from various domesticated animals. During the course of its confinement the dog developed piroplasmosis. Prior to the attack, which ended fatally, ticks had been placed on the dog, the ticks having been obtained from the vicinity of Cape Town. Some of these ticks were derived from salted or immune dogs, others from a dog affected with piroplasmosis.

Lounsbury (1901, p. 9) moreover reported 5 experiments on dogs, of which I append the following abstracts:

EXPT. I. *Irish terrier pup.* 23 Sept. 1901. Ten adult ticks (sexes equally divided) were placed upon the pup, which was kept isolated, and fed on condensed milk. 29 Sept., 20 more adult ticks were placed on the dog (10 ticks of each sex). 4 Oct., that is 12 days after the first lot of ticks were applied, the dog's temperature rose, and parasites were found in its blood. The dog remained active and showed good appetite until 8 Oct., when it showed signs of prostration. The dog died 9 Oct., that is on the 5th day after the onset of symptoms.

EXPT. II. *Adult dog.* 23 Sept. 1901. Ten adult ticks (sexes equally divided) were placed on the dog. 4 Oct., that is 11 days later, the dog's temperature rose, parasites being found in its blood. Fever continued for five days, then subsided, to return two days later, the dog becoming very ill. The dog was found dead on the morning of the 15th, that is 11 days after the onset of symptoms.

Two control dogs chained near to the preceding dog in the same shed, remained healthy, obviously for the reason that the infected ticks had not migrated to them from the animal upon which they had been placed.

EXPT. III. *Dog.* Showed distaste for food 21 days after application of infected adult ticks. On the 24th, refused food, on the 30th day parasites were found in its blood by Robertson. Diagnosis confirmed by autopsy.

The two following experiments were made on dogs, first with larvae, then with nymphs and adult ticks (Lounsbury, pp. 7—8). The ticks were derived from two blood-gorged females (*H. leachi*) found on the preceding dog (III.) after death.

EXPT. IV. 15—20. vi. Larvae placed on the dog dropped off after a few days. After undergoing metamorphosis into nymphs these

25. vii. Nymphs were placed upon the same dog. The nymphs dropped off, were allowed to undergo metamorphosis into adults (took three weeks).

The dog remained healthy.

27. viii. Adult ticks were now placed on the dog. (This dog was probably bitten also by a second batch of larvae placed on Dog V. in the same pen, but which migrated to Dog IV.) Nothing noticed until

9. ix. Dog's temperature 105° F., appeared well, parasites in its blood. 12 hours later both this dog and No. V. became prostrated, showed haemoglobinuria.

10. ix. Dog dead.

EXPT. V. 15—20. vi. Dog kept in same cage as No. IV. It was probably bitten by the larval ticks applied to the latter. Dog V. was isolated when the nymphs were applied to Dog IV. (25. vii.), and again isolated (31. vii. to 3. viii.) when a new lot of infected larvae from another source were applied.

27. viii. Some of the adult ticks placed on Dog IV. on this date wandered on to Dog V.

(28. viii. Nymphs derived from first lot of larvae placed on Dog IV. were put on Dog V.)

9. ix. Fell ill with same symptoms as Dog IV. (*q.v.*).

10. ix. Dog dead.

The negative results obtained in Experiments IV. and V., conducted with larvae and nymphs of *Haemophysalis leachi*, obtained from eggs laid by ticks removed from dogs suffering from piroplasmosis, led Lounsbury to conclude that *Piroplasma canis* is transmitted through the parent tick to its progeny. He wrote at the time (p. 10) that "such progeny normally remains incapable of transmitting the infection it inherits until it attains the adult stage. That the infection passes through the egg stage is a fact not unparalleled, and therefore not surprising; but that it is harboured through two feeding stages without being transmitted, is at present wholly unique, and is a fact that considerably strengthens the inference that in this disease and redwater, the ticks are not merely carrying agents but are secondary hosts of the protozoan parasites."

9. Immunity.

Animals affected: Robertson (1901, p. 332), has failed to infect other animals than the dog. He injected these animals with blood derived from a diseased dog, the blood showing parasites on microscopic examination. His results were negative with the following animals: horse, ox, sheep, cat, rabbit, guinea-pig, rat, mouse, fowl.

Similarly, Nocard and Motas (iv. 1902, p. 275), experimenting with the French disease, failed to infect the horse, ox, sheep, goat, cat, rabbit, guinea-pig, white rat, white mouse, fowl and pigeon. They never found parasites in the blood corpuscles of these animals although they received intravenous, intramuscular, and subcutaneous injections of infected dog's blood.

Influence of Breed and Age on Susceptibility: According to Hutcheon (1893), writing of the disease in South Africa, "imported and well-bred dogs are more liable to become affected than Colonial bred ones, but the latter become affected also. Dogs of all breeds appear subject to it." Robertson (1901, p. 327) says the disease attacks Cape bred and imported dogs indiscriminately, and (p. 336) that it affects both old and young dogs, as proved by inoculation. The South African observers do not state that young dogs are the more susceptible (see footnote, p. 242).

In France, Nocard and Motas (iv. 1902, p. 275) found young dogs to be much more susceptible than adults. Experimenting with puppies aged 2—12 weeks, they found not only that they were more readily infected, but that in them the disease ran a more acute course and that the termination was invariably fatal.

Immunity after Recovery: Both in the South African and French disease immunity has been seen to follow upon recovery. Thus, Lounsbury (xi. 1901, pp. 11, 12), in Africa, seems to have observed immunity, for he writes "one is inclined to infer the probability of practically continuous infestation by ticks being a necessary adjunct to complete immunity, as is known to be actually the case in redwater." Immunity would not appear to last long in the absence of "continuous infestation by ticks." Lounsbury cites the case of a dog which died from the disease having suffered from it two years previously.

The immunity which follows on recovery was tested experimentally by Nocard and Motas (iv. 1902, p. 277), in the French disease, on dogs which had passed through the disease both naturally or artificially

acquired. They inoculated large quantities of virulent blood into five such dogs after periods of time of 2, $2\frac{1}{2}$, $2\frac{1}{2}$, 3, 6 months respectively after recovery and found the animals in all cases to resist infection, control animals dying in every case.

It is worthy of note that in "salted" dogs in South Africa *the parasites evidently persist many months in the blood* after apparent recovery as proved by inoculations practised with their blood on fresh dogs. Thus Robertson (5. vi. 1902, p. 682) found that the fresh blood of a salted dog (No. 11), in which no parasites could be found by painstaking microscopic examination, was nevertheless capable of setting up infection in a fresh dog. The term "salted" is applied to dogs which have recovered from the disease and have acquired immunity. The salted dog had had the disease in the latter part of October. This dog's blood was used for inoculating fresh dogs as follows:

No. of dog inoculated	Date of inoculation with blood freshly obtained from salted dog	Amount of blood of salted dog injected in c.c.	Result
1	4 November, 1901	6	Dead in 10 days
2	17 " "	4	" 17 "
3	26 " "	4	" 8 "
4	3 December "	2	" 19 "
5	17 " "	6	" 13 "
6	6 January, 1902	7	Dead after many weeks
7	24 " "	5	Dead in 15 days
8	3 February "	6	" 18 "
9	25 " "	20	" 8 "
10	27 " "	27	" 3 " (from shock)
11	13 March "	2	" 34 "

These experiments show very conclusively that the blood of an apparently recovered dog may contain parasites for months (4 Nov. to 13 March) and there is no evidence that the parasites had become attenuated, for the last dog, which died on the 34th day after inoculation, it will be noted, only received 2 c.c. of the salted dog's blood, the smallest amount injected in the whole series excepting Dog 4. It is not impossible that the parasites may however become attenuated with time, but this remains to be proved. Dog 6 in the above table (constructed by me from Robertson's protocols) is obviously identical with the dog whose temperature etc. is recorded in Chart V, and for which I am indebted to Mr Lounsbury.

This persistence of virulent parasites, in the blood of apparently recovered or "salted" animals, has its parallel in what has been

observed in Texas fever by Schroeder (1900, pp. 42—43)¹ in the United States, only he has shown that the parasites (*Piroplasma bovis*) may be harboured for a very much longer time, namely from 1 to 6 years (!).

That the parasites may become attenuated is suggested by the observations of Nocard and Motas (1902, p. 273), who state that the blood of dogs suffering from the chronic disease is less virulent than in acute cases. Even allowing for the small number of parasites present in blood from chronic cases, large doses thereof usually produced but a mild form of disease. In one series of their experiments, moreover, the blood of a convalescent dog (parasites present) produced but a mild disease when injected into fresh dogs, all of the latter recovering.

Nocard and Motas made a number of observations which possess very considerable interest in relation to immunity. They found (pp. 278—281) the blood of immune dogs germicidal, for on mixing virulent blood with the serum of a recovered dog and injecting the mixture into a fresh dog, they failed to infect the animal. Such injections did not however confer immunity on dogs for they died as quickly as control animals, that is, 12 to 15 days after subsequent inoculation with virulent blood. Virulent blood and the serum of a recovered animal injected separately resulted in a fatal infection. Evidently then the immune-serum must act directly and in a certain concentration to be effective. The germicidal power of the immune-serum, absent in normal dog serum, was destroyed by exposure for 30 minutes to a temperature of 56° to 57° C. The germicidal power of the serum was greatly increased in recovered dogs which had been subjected to repeated inoculations with virulent blood.

Artificial Passive Immunity. Nocard and Motas (pp. 281—283) found that small doses of recovered dog's serum were not obviously protective, but large doses were markedly so, when injected 24—48 hours before infection with virulent blood². The immune-serum may retard the progress of the disease or completely check it. Much more potent was the serum of hyperimmunified animals, namely dogs treated with virulent blood inoculations after recovery. Here 3—5 c.c. of their

¹ Schroeder, E. C. (1900). A note on the persistence of the Texas fever organism in the blood of cattle. 16th Annual Report of the Bureau of Animal Industry for the Year 1899. U.S. Dept. of Agric., Washington, pp. 42—43.

² Carrington Purvis (cited by Hutcheon, 1899, p. 401) was the first to recommend a trial of this mode of preventive treatment, which is similar to that which has been tried in the case of Texas fever.

serum sufficed to protect dogs, control animals dying in 6 days. The immune-serum was still protective after having been heated to 56—57° C. A much smaller dose (p. 284) of heated immune-serum was required for immunization when it was mixed with virulent blood prior to injection. Infected erythrocytes exposed to the action of immune-serum, and then washed, were also capable of conferring immunity when injected; thus, in one experiment 20 drops of virulent blood were mixed with 50 drops of immune-serum and allowed to stand, being injected after they had been centrifugalized thrice and washed twice. Blood rendered avirulent by heat did not afford any protection.

The passive immunity obtained in the manner above described was of but brief duration. Whereas dogs receiving immune-serum injections 24 hours before those of virulent blood remained well, an inoculation practised 11—35 days after injection of immune-serum resulted in infection, the dogs showing few parasites in some cases, dying in others.

The serum of a sheep treated with virulent dog's blood developed germicidal properties, although the animal proved naturally immune to infection. The serum of the treated sheep did not afford any distinct protection when injected into dogs.

Immune-serum (p. 285) was proved to also possess curative properties when obtained from hyperimmunified dogs, and injected 24—42 hours after infection with virulent blood. The immune-serum was ineffective once parasites had appeared in the infected dog's blood.

10. Treatment.

Apart from the specific treatment recorded above, there is very little to note regarding treatment. In South Africa, Hutcheon (1893, p. 477, and 1899, p. 400) recommended the use of repeated doses of ammonium chloride and belladonna, a form of treatment tried by Borthwick at Port Elizabeth with "excellent results." Subsequently Hutcheon obtained encouraging results from the use of quinine, benzoate of soda, and carbolic acid. Robertson (1901, p. 332) states that he has tried quinine, calomel, ammonium chloride, extract of belladonna, carbolic acid, and finally benzoate of soda without satisfactory results. Carbolic acid appeared in fact to hasten death. He obtained the best results from a "calomel pill to start with, then a calomel and quinine pill four times a day." Without stating the dose, he says that very large amounts of calomel are needed. Hutcheon does not appear to approve of the calomel treatment. In other words the

evidence as to treatment in South Africa appears to be somewhat contradictory.

In Europe, Piana and Galli-Valerio (1895) attributed the recovery of the one dog they saw suffering from Piroplasmosis to the use of quinine. Almy (10. x. 1901, p. 379) treated his dogs with quinine bromhydrate, but observed no effect therefrom, the remedy being as ineffective as quinine has been shown to be in the treatment of Tristeza (*Piroplasmosis bovis*).

Evidently there is no known remedy for canine Piroplasmosis, and it is open to question whether or no the dogs which have been successfully treated would not have recovered anyhow.

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So as to avoid overloading the text I have omitted to mention my authorities for the statements I have made regarding this disease. Some 250 papers have appeared on the subject, most of these are given in the above bibliographies. For the reason that no complete bibliographies exist for the following diseases, I give them as follows:

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¹ I am indebted to Prof. Galli-Valerio for kindly sending me reprints of these otherwise inaccessible papers.

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Postscript.

Whilst this paper was in the press I had an opportunity of consulting a further publication by Wilson and Chowning (2. 1. 1904. "Studies in Pyroplasmosis hominis ['Spotted Fever' or 'Tick Fever' of the Rocky Mountains]."*Journ. of Infectious Diseases*, vol. 1., No. 1, pp. 31—57. Map, Charts, 2 Plates). The coloured Plate shows parasites stained by modifications of the Romanowsky method and the figures are much more convincing than those which appeared in the earlier paper by these authors, cited by me on p. 221. We may therefore accept the name (*Piroplasma hominis*) which they give to the parasite.

Wilson and Chowning (p. 52) cite H. P. Johnson (*Proc. Am. Veter. Med. Assoc.* 1903) as having observed *Piroplasma ovis* in sheep suffering from "pyroplasmatic ictero-haematuria" in Deer Lodge Valley, Montana, U.S.A.

caused onset of fever on 16th day. 11 ticks put on after onset (18th day) so that they might become infected from the dog. First ticks began dropping 13th day. Dog off his feed 18th day. Very weak 23rd.

11

20

20

No. of infected ticks put on

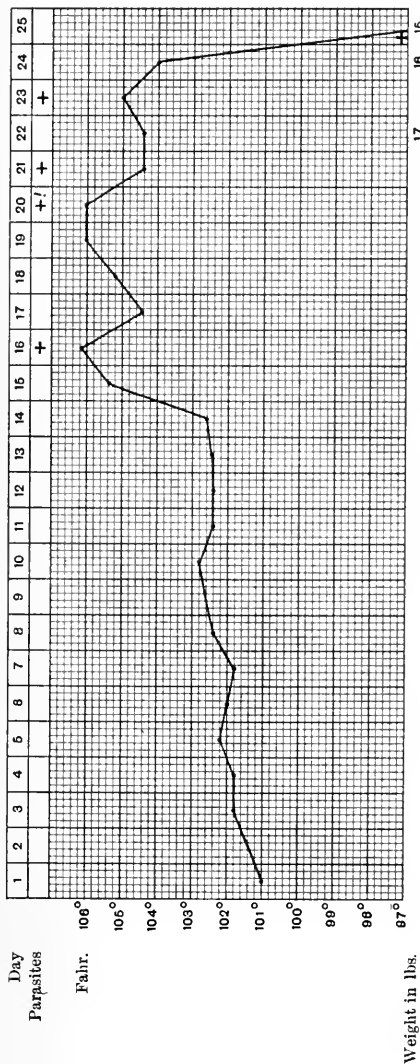


CHART II. Tick infection. Dog 5. Fox-terrier (long-haired). 26 ticks placed on dog on days 1, 7 and 12, the last near the onset of symptoms. Appetite poor 14th, food refused 19th and after, when grew rapidly weak.

† No. of infected ticks put on

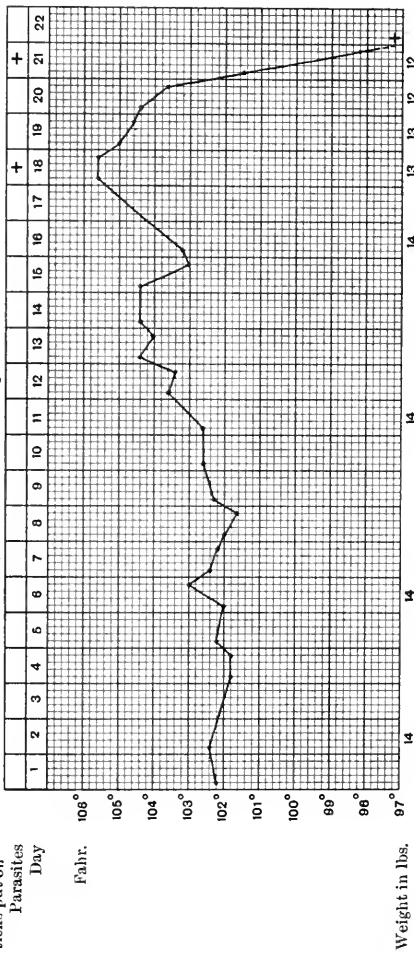


CHART III. Tick infection. Day 7. Mongrel terrier, aged, long-haired. 22 ticks, sexes equally divided, were put on on days 1, 4 and 11; and 6 more ticks were put on on day 16 when dog showed fever. Dog ceased feeding 17th day until death 23rd day.

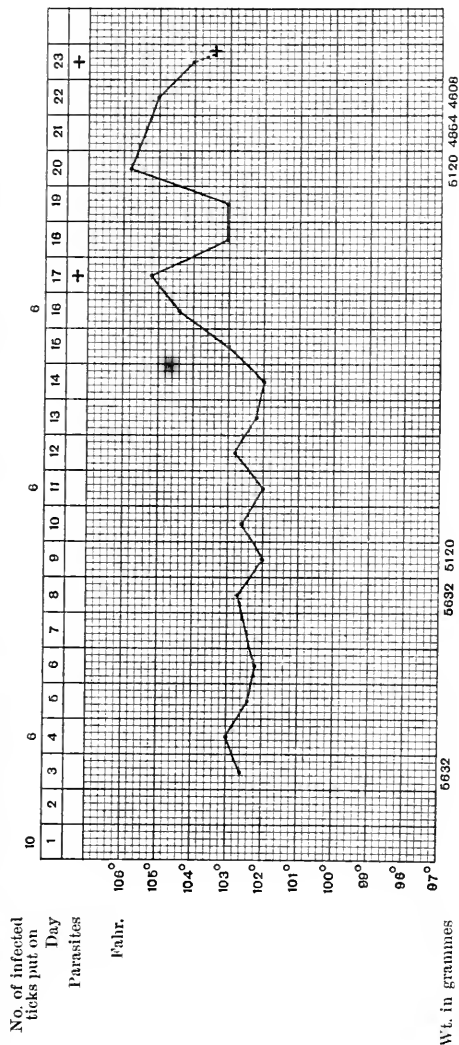


CHART IV. Infection by subcutaneous inoculation. Day 3. Bitch (Fox- and Bull-terrier cross). Subcutaneous inoculation with blood (6 c.c.) of Day 1 found dead on morning of 1st day. On the 10th day (after onset) 20 ticks placed on bitch so that they might become infected. 11th day bitch off her feed; 12th refused food, weak; 13th weaker, mucous membranes pale; 14th tottering, haemoglobinuria, rectal temp. 90° F., sinking.

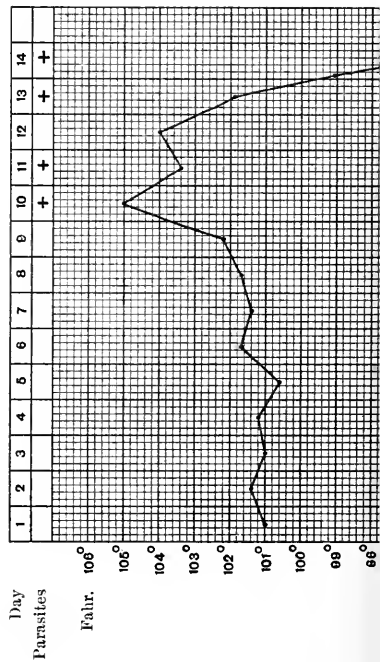


CHART V. Infection by subcutaneous inoculation. Chronic type. *Mr Lounsbury's Case. Small adult male dog (No. 17) inoculated 7. I., died 16. III. 1902. Received 7 c.c. of citrated blood (under shoulder) fresh drawn from Dog 11, recovered animal, sick in Oct. 1901. Fed on dog biscuit and condensed milk during illness. Fever began 7th day, died 62nd day of illness, no haemoglobinuria throughout. (Through an oversight the sign + was omitted in the parasite column on days 11—14, 28, 30, 31.)*

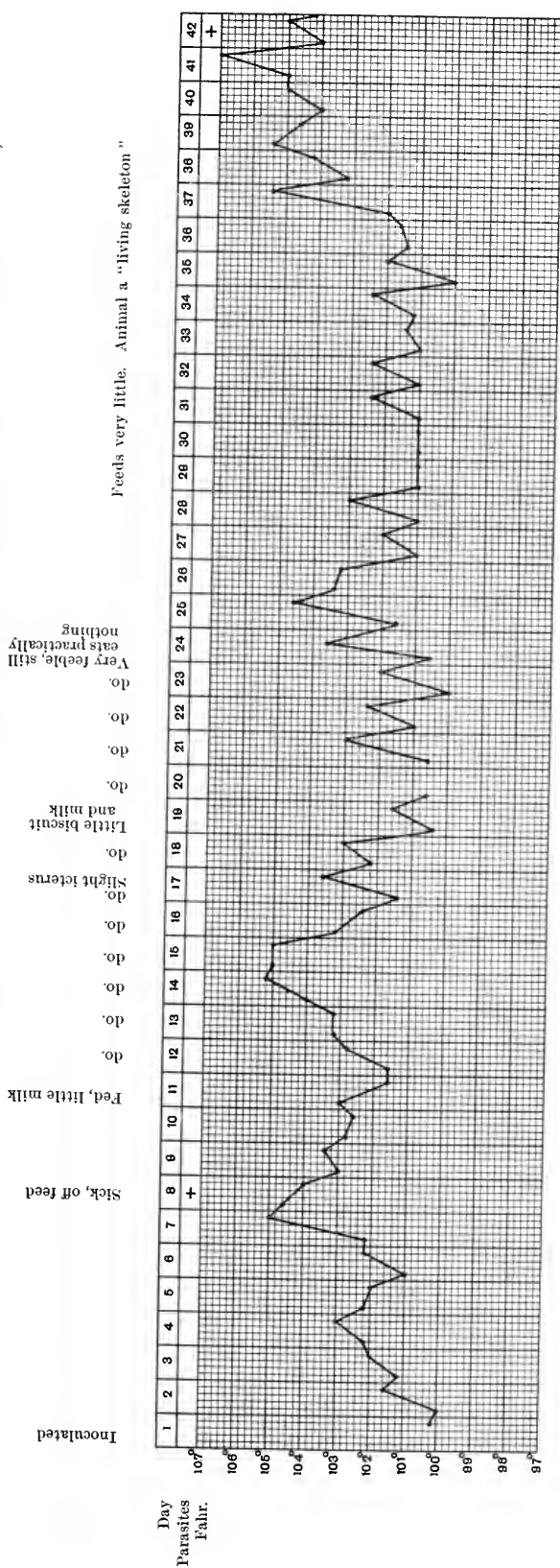


CHART V. (continued)

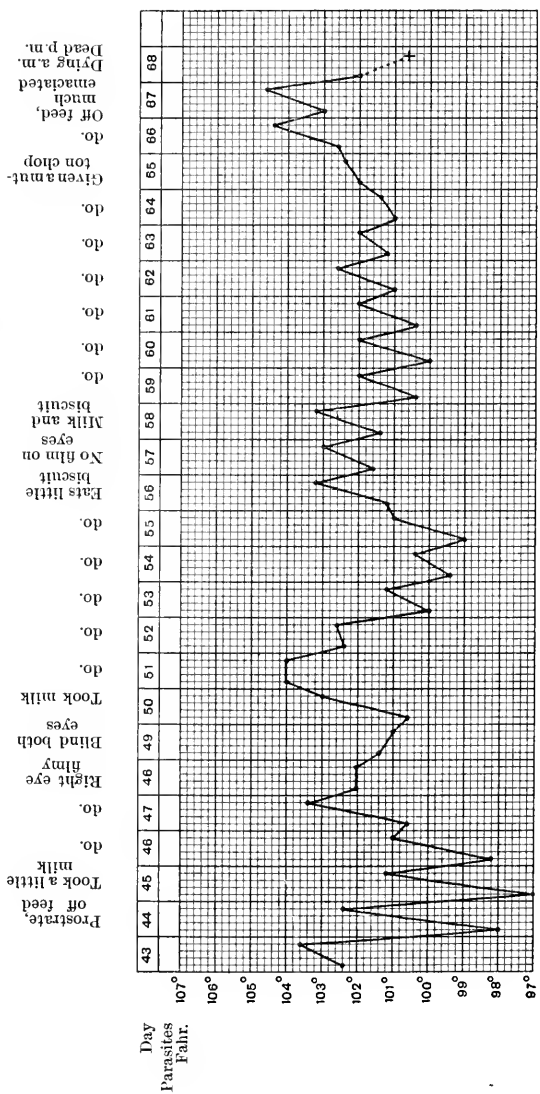


CHART VI. Infection by intravenous inoculation. Combined temperature chart for two dogs which received an intravenous inoculation of 5 c.c. of the blood of a naturally infected dog. After Robertson (1901, pp. 328, 329.) Death on the 8th day after injection.

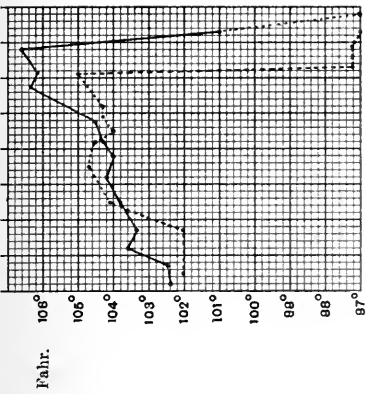
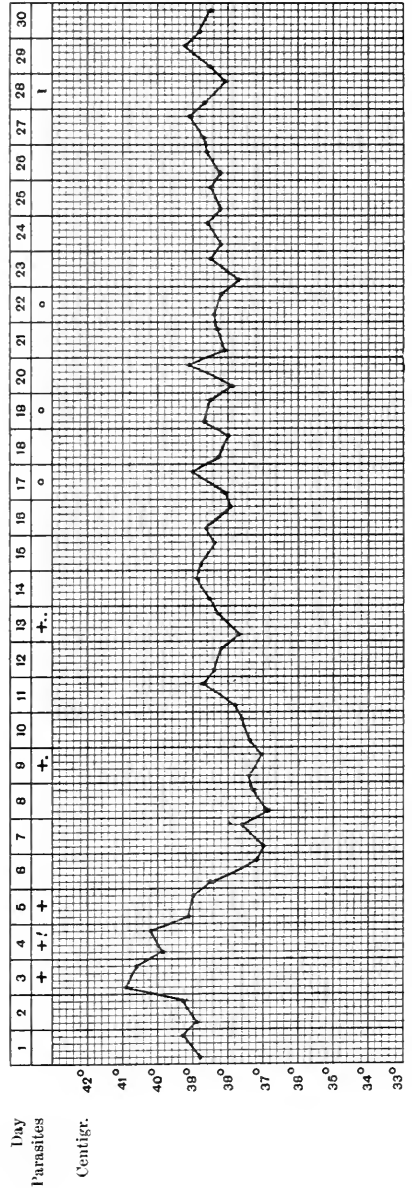


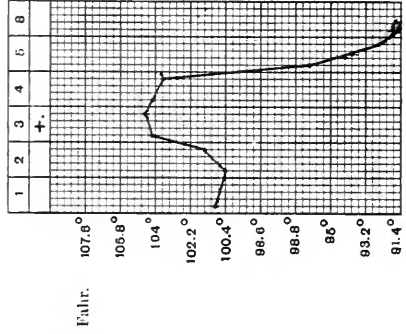
CHART VII. Infection by intravenous inoculation. Case I. chronic, II. acute. French canine piroplasmosis. After Nocard and Mutas (1902, pp. 288, 289). The blood counts etc., are given on pp. 238—239.

Case I. Parasites & fever 3rd day. Case ended in recovery.

Parasites { + moderate
 ++ numerous
 +. few
 +. very few
 1 single one found
 0 none found



Case II. Acute, death during night of 5—6th day



A STUDY OF THE VIRULENCE OF THE DIPHTHERIA
BACILLI ISOLATED FROM 113 PERSONS, AND OF
11 SPECIES OF DIPHTHERIA-LIKE ORGANISMS, TO-
GETHER WITH THE MEASURES TAKEN TO CHECK
AN OUTBREAK OF DIPHTHERIA AT CAMBRIDGE, 1903.

By G. S. GRAHAM-SMITH, M.A., D.P.H., M.B. (CAMB.).

*(From the Pathological Laboratory of the University
of Cambridge.)*

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DIPHTHERIA BACILLI FROM SERUM CULTURES.

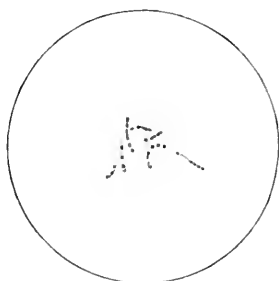


Fig. 1.
Irregularly beaded form.
Subculture 24 hours.

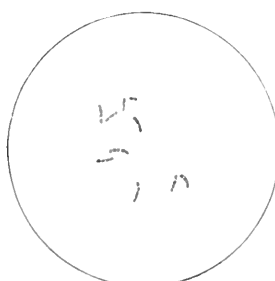


Fig. 2.
"Streptococcal" form.
First culture 24 hours.

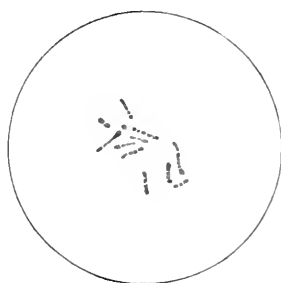


Fig. 3.
Segmented form.
First culture 24 hours.

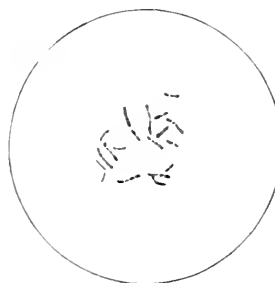


Fig. 4.
Segmented form.
First culture 24 hours.

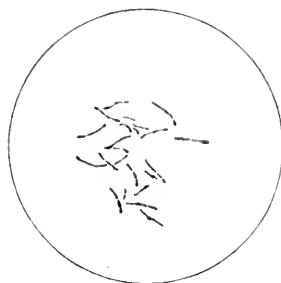


Fig. 5.
Thin segmented form.
First culture 24 hours.

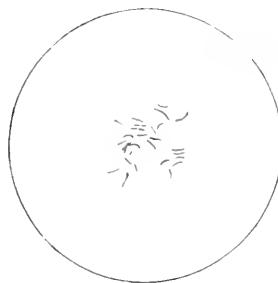


Fig. 6.
Uniformly stained form.
Subculture 24 hours.

All figures drawn with the aid of a camera lucida (Zeiss $\frac{1}{12}$ in., No. 4 oc.)
stained with Löffler's methylene blue (diluted 1 : 5).



DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

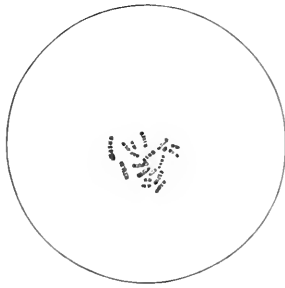


Fig. 7.

Hofmann's bacillus (Pseudodiphtheria type). First culture 24 hours.



Fig. 8.

Typical Hofmann's bacillus. Subcultures from Pseudodiphtheria type 24 hours.

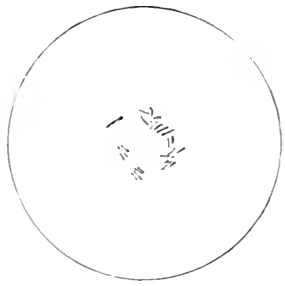


Fig. 9.

Bacillus diphtheroides citreus. First culture 24 hours.

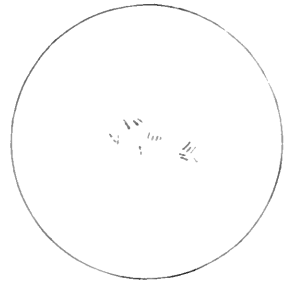


Fig. 10.

Bacillus diphtheroides brevis. First culture 24 hours.



Fig. 11.

Bacillus maculatus. First culture 24 hours.



Fig. 12.

Bacillus diphtheroides liquefaciens. First culture 24 hours.

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DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

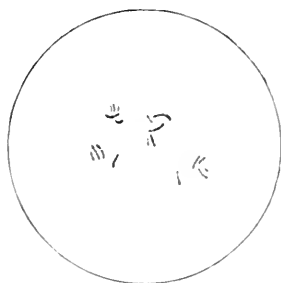


Fig. 13.
Bacillus xerosis.
First culture 30 hours.



Fig. 14.
Bacillus xerosis canis.
First culture 48 hours.

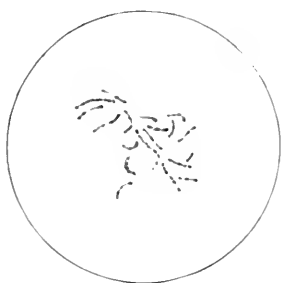


Fig. 15.
Bacillus auris.
First culture 48 hours.



Fig. 16.
Bacillus ceruminis.
First culture 30 hours.

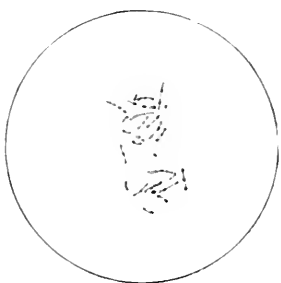


Fig. 17.
Bacillus diptheroides gallinarum.
First culture 24 hours.

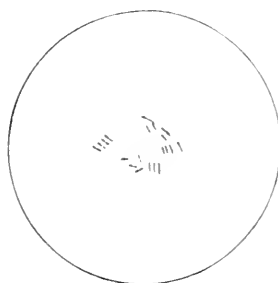


Fig. 18.
Bacillus cuculi.
First culture 12 hours.

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COLONIES ON ALKALINE POTATO AGAR.

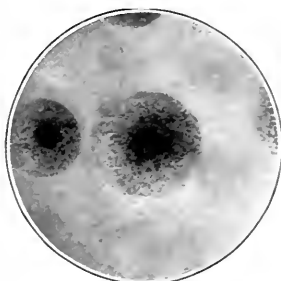


Fig. 1.
B. diphtheriae. Type (a).
48 hours.



Fig. 2.
B. diphtheriae. Type (a), rare form.
48 hours.



Fig. 3.
B. diphtheriae. Type (b).
48 hours.



Fig. 4.
B. diphtheriae. Type (b).
48 hours. Drawn under Zeiss'
binocular dissecting microscope.
A.o. lens. No. 4 oc.



Fig. 5.
B. ceruminis. 48 hours. The majority
of diphtheria-like organisms includ-
ing Hofmann's bacillus produce
colonies of this type.



Fig. 6.
B. maculatus. 48 hours.

These colonies were (except Fig. 4) all photographed by Mr Walter Mitchell of this Laboratory: Fig. 1 by direct and the others by oblique illumination, all of the same magnification.

•

DURING the spring of 1903 an outbreak of diphtheria occurred amongst the children attending certain schools in Cambridge. The measures taken to check this outbreak included the bacteriological examination of all notified persons and contacts, and the isolation of such as were found to be harbouring diphtheria bacilli in their throats. These measures depend on the observation that a certain number of persons, who have come into contact with cases of diphtheria, become infected with the specific organism, and, while remaining healthy, are liable to give the disease to others. The bacteriological efforts to check the disease are also based on the assumption that *virulent* diphtheria bacilli do not occur in the mouths and noses of healthy people, who have not been in some way exposed to persons suffering from the disease, or persons who have acquired the bacillus by being so exposed. This view is not, however, held by all the authorities on the subject, for while some are of the opinion that virulent diphtheria bacilli are never to be found in the throats of healthy persons, who have had no opportunity of acquiring the bacillus by contact, others believe that it does occur in small numbers amongst the normal population.

Should the view of the latter school be accepted, the method of attempting to suppress epidemics by the isolation of healthy infected individuals is not only likely to prove unsuccessful, but entails unnecessary hardship on the isolated persons.

Before proceeding to the results of measures adopted in this instance, a short summary of some of the investigations, on which the principles depend, is given.

*The occurrence of diphtheria bacilli in notified persons*¹.

The results of the bacteriological examination of nearly 27,000 persons certified on clinical grounds to be suffering from diphtheria by English, Continental, and American investigators show that in 72 % organisms morphologically resembling diphtheria bacilli are present. Virulence and cultural tests of the bacilli found have only been made in a very small proportion of cases.

¹ For a more detailed account of the investigations summarized in these paragraphs see Graham-Smith (1903, pp. 217—219), and the original papers there referred to.

*The occurrence of diphtheria bacilli in persons who have come in contact with cases of the disease, or with others who have acquired the bacillus in this way*¹.

All observers are agreed that virulent and dangerous diphtheria bacilli occur in the mouths of certain healthy persons who have come into contact with the sick, or with others who like themselves harbour the organisms.

The proportion of infected to non-infected contacts is subject to great variation according to the investigations of different observers. To some extent these differences probably depend on the measures taken to promptly isolate the sick, the class of persons examined, and the views of the observer as to the importance of the bacilli which he finds.

Table I gives the results of several investigations on this subject.

TABLE I.

Name of observer	No. of persons examined	No. of infected persons found	Percentage of infected persons
<i>Infected Families.</i>			
Cobbett (iv. 1901, p. 231)	9 ?	9 ?	100
Spirig (1899)	9	6	66·6
Park and Beebe (1894)	48	24	50
			} 59
<i>Hospital Wards.</i>			
Lister (1898)	125	61	48·8
Müller	100	14	14
Park and Beebe (1894)	55	6	10·9
Chatin and Lesieur (1900)	75	2	2·6
			} 23·4
<i>Schools.</i>			
Goadby (1900)	600	190	34·1
Peck (1901)	100	31	31
Berry and Washbourn (1900)	142	17	11·9
Denny (1900)	200	22	11
Graham-Smith (1902)	519	54	10·4
			} 20·1
<i>General Contacts.</i>			
Meade Bolton (1896)	214	95	45·5
Kober (1899)	128	15	11·7
Cobbett (iv. 1901, p. 242)	650	19	2·9
			} 13

The figures which have just been quoted emphasise the fact that a very considerable proportion of the persons exposed to the disease

¹ See note p. 259.

acquire diphtheria bacilli without signs of illness. The proportion is highest amongst the closely related persons in the infected family, and gradually diminishes as the opportunities of contact become less frequent.

The occurrence of virulent diphtheria bacilli in persons who have not recently been in contact with the disease¹.

Reliable observations on this point are very few, since most of the investigators have not isolated and tested the organisms they have found, but have depended on their morphology in culture alone, nor have they usually made any special inquiries into the possibility of recent contact.

Observations of this kind can only indicate in what proportion of persons organisms more or less resembling diphtheria bacilli occur, but no conclusions as to their power of transmitting the disease can be made. Nevertheless some observers have drawn far-reaching conclusions from the results of these experiments, even to the extent of stating that one out of every seven normal children amongst the community harbour diphtheria bacilli in their throats, and are therefore a possible source of danger.

The results of observations, in which morphology in culture alone was in most cases relied on, are given in the following table.

TABLE II.

Observer	No. of persons examined	No. of persons in whom organisms morphologically resembling diphtheria bacilli were found
Herman Biggs	330	32
Garratt and Washbourn (1899)	666	8
Hewlett and Murray (1901)	385	58
*Massachusetts Committee (1902)	1993	10
	3374	108 (3·2%)

* In their table the authors of this report include persons examined at Boston, New York and Brooklyn with the places from which came the persons here recorded. Owing to the statement that diphtheria was prevalent in these cities I have excluded them.

It is very noticeable in such statistics that, whenever the virulence of the organisms discovered has been tested, a large proportion has been found to be non-pathogenic. In the above list 26 (74%) out of the 35 tested turned out to be devoid of virulence.

¹ See note p. 259.

The following table on the other hand shows the results of observations on normal throats in which careful inquiries were instituted as to the possibility of recent infection, and all suspicious organisms isolated and tested for virulence.

TABLE III.*

Observer	No. of persons examined	No. of persons harbouring non-virulent diphtheria bacilli	No. of persons harbouring virulent diphtheria bacilli
Park and Beebe (1)	324	24	2
Kober (1899)	590	5	0
Denny (1900)	235	1?	0
Graham-Smith (1903, p. 252)	362	1	0
	1511	31 (2·05 %)	2 (·13 %)

* Persons found on inquiry to be recent contacts have been excluded from this table.

The investigations of these observers have shown that in a large proportion of their cases, in which bacilli morphologically and culturally identical with diphtheria bacilli have been found, they have been devoid of virulence. On further inquiry amongst those who have harboured virulent bacilli they have elicited the fact that the latter were, in almost every case, recent contacts. After eliminating all such recent contacts it was found that virulent diphtheria bacilli occurred, and could not be satisfactorily accounted for, in two persons out of 1511. In a third person bacilli morphologically identical with diphtheria bacilli were discovered, but were too few in number to allow of a pure culture being obtained (see Denny).

Remembering the great difficulty often met with in prosecuting inquiry amongst school children, and the class of persons from whom hospital cases are drawn, and amongst whom these investigations were principally conducted, these figures are very striking, and in the absence of further evidence undoubtedly point to the conclusion that virulent diphtheria bacilli are seldom, if ever, present in the throats of healthy persons, who have not recently been in contact with cases of diphtheria or infected contacts.

The results of the isolation of infected contacts¹.

The attempts which have already been made to stamp out diphtheria outbreaks by the examination of contacts, and the isolation of those found to be infected, have been attended with encouraging results.

¹ See note p. 259.

They have principally been made in isolated schools and institutions in the towns of Minnesota by the State Board of Health (1900), and by several observers in this country. More extensive outbreaks involving several schools in a town have been similarly treated also with a considerable measure of success by Cobbett (IV. 01, and X. 01) at Cambridge and Graham-Smith (1902) at Colchester.

Cobbett (XI. 1901) consequently considers that "the duty of discovering, isolating, and disinfecting the former class of persons (*i.e.* infected contacts) is becoming more and more the urgent duty of the Sanitary Authorities. For the fact that they are not scattered broadcast throughout the community as was once supposed, but are confined to the class of persons whom we conveniently call 'contacts,' renders their discovery a practical possibility, and offers a fair prospect that at least the great majority of them may in the near future be subjected to isolation and antiseptic treatment to the immense advantage of the public health."

The measures adopted in this epidemic.

(1) Cultures were examined from all notified persons, and all those in whose throats diphtheria bacilli were found were isolated until three consecutive negative examinations showed them to be free from the bacilli. The majority were isolated in the infectious diseases hospital and the others at their own homes.

(2) As far as possible all children belonging to families in which cases of diphtheria had occurred, and all persons known, or likely, to have been in contact with the cases were also examined, and, if diphtheria bacilli were found in their throats, isolated. Particular attention was paid to the schools in this respect. Whenever a case occurred the class which the child attended, and on one occasion nearly the whole school, was examined. The parents of the infected children were informed of the fact, the dangers were explained to them, and in most cases their consent was readily obtained to the isolation of the children in an Isolation Home. In the few cases in which the parent's consent could not be obtained the children were kept at home, and not allowed to attend school. In all these cases three consecutive negative examinations were required before release from isolation.

(3) As far as possible all cases of sore throat brought to the notice of the medical practitioners, and more especially those occurring among school children, were investigated.

(4) The means by which the disease is generally considered to be communicated to others by patients and contacts were explained to the school teachers, and precautions taken to guard against its spread by infected articles.

(5) The administration of antitoxin as a prophylactic to healthy contacts, who showed the bacillus in their throats, was encouraged, as well as the use of antiseptic mouth washes.

The work of carrying out all these administrative measures entirely rested with Dr Bushell Annington, the Medical Officer of Health, and to him belongs the credit of the great measure of success which attended them.

General history of the epidemic.

The epidemic practically resolves itself into a series of outbreaks in various schools and institutions, most of which promptly ceased when the measures just mentioned were put in force.

The outbreak commenced in the middle of March 1903, when several scholars attending the Infants' Department of the St Matthew's School were notified to be suffering from diphtheria. After the examination of the school and the isolation of infected contacts, it was closed for other reasons, but some cases occurred amongst its scholars, and persons connected with them, probably by means of untraced contacts. In the Girls' Department two cases were notified about the same time, and examination revealed several healthy contacts. No further cases occurred in this department. In the Infants' Department, however, a recrudescence occurred in November, probably by means of an infected contact from the March outbreak. This child had been isolated at that time and released after three consecutive negative examinations. During the November outbreak he was again found amongst the infected contacts. On both occasions the bacilli isolated from this child were found to be virulent.

One of the first cases to be notified from the Infants' Department was isolated in the Hospital. By this means apparently several persons became infected. The type of the disease was, however, very mild, and most of the six notified persons had only slight clinical signs. This outbreak was promptly brought to an end by the examination of the patients in the infected ward and the attendants, and the isolation of those found to be harbouring the specific bacillus.

At the Sturton Street School one child was notified on March 24th.

This patient lived only a few doors from some of the infected contacts found in the St Matthew's School, and it seems likely she became infected from some of them.

The examination of the scholars only revealed one infected healthy child. In May two scholars of this school suffered from the disease, but it is extremely probable that they were infected in the same manner as the original case. No other cases occurred in this school.

At the Sanatorium a curious instance of the spread of the disease was noticed amongst Scarlet Fever cases¹. One of these was found to have virulent diphtheria bacilli in a discharge from the ear. The examination of the other patients showed that five of them had diphtheria bacilli in their mouths. Three cases probably infected from this source were notified. After the discovery of the condition that existed, and the separation of the infected persons, no further cases occurred.

In November an outbreak appeared in the Catherine Street School, which was immediately suppressed by the examination of all the scholars and the isolation of the infected ones.

Besides the outbreaks just mentioned some isolated persons, in whom the source of infection could not be traced, were notified. Many of these only showed slight clinical signs, and were only certified on the finding of diphtheria bacilli. Further investigation in a number of the cases showed that the bacilli were non-virulent (pp. 270 and 284).

Detailed account of the results of the examinations of notified cases and contacts, and of the virulence of the diphtheria bacilli found.

St Matthew's School. (Girls.) The first case to be notified in which diphtheria bacilli were found was that of a child (1)² attending the Girls' Department of the St Matthew's School on Feb. 20th. Two examinations were made at this time but no diphtheria bacilli were found, but during the examination of the healthy children in this school in March, this child was found to be harbouring virulent diphtheria bacilli. It is of course possible that her illness was not

¹ For details see p. 268.

² The numbers refer to the tables at the end of the paper showing the period during which the bacilli were found in the mouth, the presence or absence of Hofmann's bacillus, the virulence of the diphtheria bacilli, and the results of the autopsies of the inoculated animals.

due to diphtheria bacilli, but to the streptococci which were found in large numbers, and that she subsequently became infected with diphtheria bacilli, but on the other hand it seems more likely from subsequent events that she was at first really infected with diphtheria bacilli, which were for some reason not discovered, and that her case was the starting point of the outbreak. Early in March another case was notified, but although examined on many occasions no diphtheria bacilli were found.

Towards the end of March two children (2, 3) developed the disease. The former showed virulent diphtheria bacilli, but the latter died before any examination could be made.

Within a few days 122 children from this school were examined and 7 with diphtheria bacilli found, including the child first mentioned. Six of these (1, 5, 6, 7, 8, 9) harboured virulent diphtheria bacilli, and one (4) non-virulent bacilli. With two exceptions (1, 9) all were treated at the Isolation Home.

No further cases have occurred in this department of the school.

St Matthew's School. (Infants.) Between March 10th and 14th four scholars (10, 11, 13, 14) attending this department of the school developed diphtheria, probably being infected from children attending the other department.

The bacilli obtained from three were virulent, but those from one (10) were not tested.

By March 22nd 279 children, scholars of the school and family contacts, had been examined, and 16 harbouring diphtheria bacilli discovered. Four of these (12, 15, 24, 29) subsequently developed the disease. The latter and eight others showed virulent bacilli (16, 17, 18, 19, 20, 22, 23, 25), and four (21, 26, 27, 28) non-virulent bacilli. All the healthy contacts harbouring virulent bacilli were treated at the Isolation Home.

Two other children (33, 34), associated with scholars of this school, were notified at this time. The former was a brother of one of the clinical cases (29), and the latter lived close to several of the infected children.

In May two scholars (30, 31) developed the disease. Both these as well as four other children (35, 36, 37, 38), who were notified in June, probably acquired the infection at their homes from some undiscovered source as all lived within a small area. One child (32), a sister of one of the notified scholars (31), was also found to be

harbouring diphtheria bacilli. The bacilli from all the nine children just mentioned were virulent.

The school, which had been closed from the middle of March, was opened early in June, but no further cases occurred for five months. Then a second outbreak occurred, probably caused by one of the infected contacts of the earlier outbreak. This child (67, No. 23 March outbreak) who had been found in March to be harbouring virulent diphtheria bacilli was allowed to go home after three consecutive negative examinations. In the outbreak which occurred in November he was again found to be harbouring virulent diphtheria bacilli.

In the November outbreak four scholars (61, 62, 63, 64) and two children (69, 70) connected with the school were notified to be suffering from diphtheria. All showed virulent bacilli. The class to which they belonged containing 59 children was examined, and four infected contacts (65, 66, 67, 68) were discovered. The bacilli isolated from all these were virulent. Two of these (66, 67) were treated at the Isolation Home.

Since that time no further cases have occurred.

Hospital. One of the first cases (14) to be notified at the St Matthew's School was treated at the Hospital. In March a case (45) occurred there, and no other source of infection could be found. Apparently infected through the attendant on this patient five cases occurred (46, 47, 48, 50, 49), all of a mild type, and only notified because diphtheria bacilli were discovered. Two of these were first discovered as apparently healthy contacts. The first four showed virulent, but the other had non-virulent bacilli. Amongst the patients in the infected ward one contact (51) with virulent bacilli was found.

This outbreak then ceased, and no further cases have occurred.

Sturton Street School. Towards the end of March a child (39) attending this school was notified to be suffering from diphtheria. The bacilli were virulent. Infection probably occurred at home through contact with one of the scholars of the St Matthew's School.

114 children from this school were examined, and one (40) found with virulent diphtheria bacilli.

In May two other scholars (41, 43) developed the disease, in all probability by contact with the children (35, 38) near whose homes they lived. The brother (44) of the latter was also attacked shortly afterwards. The bacilli discovered in these three children were virulent. Non-virulent diphtheria bacilli were also discovered in one (42) of the

family contacts of the first mentioned. Both the infected contacts found in connection with this school were treated in the Isolation Home.

No further cases have occurred in this school.

Sanatorium. Amongst the patients isolated here suffering from scarlet fever a curious outbreak occurred. In June a case (54) of diphtheria occurred and another patient (52) with a sore throat was notified on diphtheria bacilli being found in the culture. The bacilli in both these cases were virulent. Shortly afterwards it was discovered that one of the scarlet fever patients (53) with a discharge from the ear had virulent diphtheria bacilli in the pus. On this 49 persons who had been in contact with him were examined, and five (55, 56, 57, 58, 59) were found to have virulent diphtheria bacilli in their throats. On means being adopted to separate these persons the outbreak came to an end.

Catherine Street School. At the end of November two clinical cases (72, 73) of diphtheria were notified. Both showed virulent bacilli.

Five classes in this school were examined. In class iii of 54 children to which these patients belonged eight infected contacts were discovered. Seven of these (75, 76, 77, 78, 79, 80, 81) harboured virulent diphtheria bacilli. Infection may have been conveyed to this school through a patient (71) who suffered from the disease in May by means of one (77) of these infected contacts. The latter lived close to the former, and had been attending the school for some time with a sore throat. In any case the bacilli had probably been in this child's throat for some time, and the high percentage of infection can be accounted for in this way. It was noted that nearly all these infected children sat close together in school. One other child (74) also had diphtheria bacilli, but they were non-virulent.

In the fourth class with 54 scholars four (82, 83, 84, 85) infected with diphtheria bacilli were discovered. In the first two the bacilli were non-virulent, but in the latter two virulent.

In two classes (i and v) with 47 and 41 children respectively, none harbouring diphtheria bacilli were found, but in the second room with 102 children one (86) was found with virulent bacilli.

The classes chiefly infected were only separated by a curtain, and presumably mingled freely.

Of the contacts harbouring virulent diphtheria bacilli all but three (75, 77, 84) were treated at the Isolation Home. Three weeks later one further case (87) occurred and the outbreak then ceased.

Probably connected with this school through contact at home was the case of a girl (88) notified to be suffering from diphtheria early in January (1904). The bacilli isolated from this case were virulent, but those from her brother (89) were non-virulent. The patient worked in a dress-making establishment from which 30 persons were examined without finding diphtheria bacilli, and no cases of the disease occurred amongst them.

It is also possible that a patient (117) attending the *Ross Street School* was infected from the above source. 47 scholars belonging to the class which this child attended, and five family contacts were examined, but no diphtheria bacilli were found.

Post Office. In February 1904 one (95) of the employees in the Post Office was found to be suffering from a sore throat. On examination virulent diphtheria bacilli were found. Within a few days another similar case (96) occurred. Amongst the family contacts of the latter two (97, 98) harbouring diphtheria bacilli were discovered. In connection with these cases 52 persons connected with the Post Office, and 19 with a printing establishment, in which one of the contacts (97) worked, were examined, and one (99) healthy contact found. All the last four persons, who were almost certainly infected from the same source, were found to have non-virulent bacilli. (Also see Addendum, p. 321.)

New Street School. Early in March 1903 a scholar from this school died from a disease which appeared to be diphtheria. A thick membrane covered the palate and tonsils. Numerous cocci were found, but neither from the exterior, nor the interior of this membrane could diphtheria bacilli be cultivated, nor could they be demonstrated in sections made from it. Forty-three children from this school were examined, and one (91) with non-virulent diphtheria bacilli discovered.

Abbey School. About the same time a scholar attending this school was notified to be suffering from diphtheria, but no diphtheria bacilli were found on culture. Thirty-three children from the school were examined, and one (90) with non-virulent diphtheria bacilli discovered.

No cases of the disease occurred in these schools.

Two children (92, 93), who had been attending the *St Barnabas School*, suffered from sore throats and virulent diphtheria bacilli were isolated. A sister of these children also showed diphtheria bacilli on culture, but the bacilli could not be isolated. No other cases of the disease occurred in this school¹.

¹ Details of the occurrence of the pseudo-diphtheria bacillus amongst the scholars of these schools are given in Table VIII.

Certified cases of diphtheria in whom the source of infection was not discovered.

During the course of the year 91 suspicious cases of sore throats were examined, and in 12 of these diphtheria bacilli were found. The bacilli in seven of them were virulent (100, 101, 102, 103, 104, 105, 106). The source of infection in the first four was not definitely ascertained, but was probably connected with one of the schools. In the last three it is quite unknown, but in the first two of these the clinical signs were very slight.

Five persons (107, 112, 113, 114, 115) were also notified on very slight clinical grounds on the discovery of diphtheria bacilli in their throats. The organisms in all these cases were devoid of virulence.

In connection with the first of these, four (108, 109, 110, 111) family contacts harbouring diphtheria bacilli were found, in all of whom they were non-virulent.

It will be seen from the foregoing account that these efforts to check the disease met with a considerable degree of success.

In five out of the six institutions in which the measures were applied, immediate, and almost complete, success was the result. In the case of the Infants' Department of the St Matthew's School the results were not so encouraging. In the first outbreak the effect of the isolation of the contacts was to check the disease for a time, no cases occurring for six weeks. Then several cases occurred in the district from which most of the children are drawn. The subsequent outbreak in November was, however, immediately controlled.

The discovery of persons infected by contact at the homes of the scholars is a matter of considerable difficulty since infection has in almost all cases occurred before the patient or contact, through whom it has been conveyed, is discovered. Consequently only inquiries at the homes can determine how many persons were liable to have been recently infected, and these must be visited at their homes to obtain swabs. It is probable that infection was conveyed to the few notified persons, in whom its source was not discovered, by such unrecognized contacts.

Certain contacts during the first part of the epidemic were isolated in whose throats suspicious organisms were found. On further investigations most of these organisms were found to be Hofmann's pseudo-diphtheria bacilli, or bacilli differing from diphtheria bacilli in

culture though resembling them in morphology. No mention has been made of such persons in the foregoing account.

In the treatment of outbreaks of diphtheria the closing of schools has seldom been found to be effectual in checking the disease, and when the methods which have been used in this case are employed, the difficulties are increased by doing so. While the school remains open the occurrence of a fresh case can immediately be followed by the examination of the school contacts, and intimate friends of the patient, and the movements of the patients before the attack can be fairly easily ascertained. If the schools are closed on the other hand the children are at liberty during the day and play with each other, so that it becomes extremely difficult to trace and examine all the possible contacts.

Of the infected contacts found during the outbreaks 39 were completely isolated in the Isolation Home, or in some other way, but the consent of the parents to the isolation of 17 could not be obtained. Fortunately, however, 10 of these harboured only non-virulent bacilli, which appear to be non-pathogenic to man (p. 286).

Although, whenever possible, three consecutive negative examinations were required before release from isolation, in a few instances it was found impossible to enforce this rule. This was especially the case in those children who could not be efficiently isolated, and in whose throats the bacilli lingered for long periods.

From 13 persons (9, 31, 45, 48, 50, 65, 68, 77, 84, 93, 94, 102, 104) with virulent bacilli two consecutive negatives were, however, obtained, and from one person (113) with non-virulent bacilli. One negative was obtained from two persons (1, 53) with virulent, and from three (108, 110, 114) with non-virulent bacilli. Four persons (32, 52, 69, 92) with virulent bacilli, and five (26, 28, 90, 109, 112) with non-virulent bacilli still showed them on the last examination.

It is worthy of note that in 43 of the examinations for the release of persons harbouring diphtheria bacilli at the time when the organisms were scarce, they were only found on looking at the culture for the second time after twelve to twenty-four hours' further cultivation.

It has been previously mentioned that 91 cases of suspicious sore throat were examined, 79 without finding diphtheria bacilli, although at least fourteen of the latter were certified on clinical grounds to be suffering from diphtheria. Of these persons 44 were examined two or three times, nine on four occasions, and two on five. Several of these certified cases subsequently developed scarlet fever.

That a not inconsiderable number of persons notified to be suffering from diphtheria, especially during epidemics, show no bacteriological evidence of the disease is the experience of the majority of observers. Woodhead (1896), for example, found that 20 % of the certified cases out of 12,172 admitted to the Metropolitan Asylums Board Hospitals during the years 1895—6 showed no diphtheria bacilli on culture, and the Massachusetts Board of Health (1900) report that during five years of the 2461 cases diagnosed as diphtheria on clinical grounds 859 (35 %) were negative on bacteriological examination, whereas out of 2977 doubtful cases with insufficient clinical signs for diphtheria 824 (27 %) were positive.

The treatment of epidemics of any considerable size by the bacteriological method, especially if all the diphtheria bacilli and doubtful organisms are isolated, involves a considerable amount of labour. During this outbreak over 2200 cultures were examined, of which 757 were re-examined after further growth, and more than 350 organisms were isolated, of which 194 were fully investigated, namely 113 diphtheria bacilli, 26 pseudo-diphtheria (Hofmann's) bacilli, and 55 diphtheria-like organisms obtained from various sources.

In all, over 7000 cultures for diagnosis, and the observance of the characteristics of the bacteria in pure culture, were examined and the results recorded.

The characters of the diphtheria bacillus.

There can be little doubt "that when once one has become fully acquainted with the range of its variation it is fairly easy to recognize the diphtheria bacillus and distinguish it from all others," but "*the eye cannot be sufficiently trained for this purpose unless the observer frequently tests the opinions he forms on morphological grounds by isolating his cultures, and testing them in various ways, including the injection of animals*" (Cobbett, IV. 01, p. 236).

In the practical diagnosis of diphtheria it must be assumed that organisms morphologically resembling diphtheria bacilli are true diphtheria bacilli, for the opinion of the bacteriologist to be of any practical value cannot await the preparation of pure cultures and the injection of animals; but if deductions of any *scientific* value are to be drawn from the observations the testing of the organisms found is essential, especially if the deductions are to be made the bases on which epidemics of diphtheria are to be combated by bacteriological means.

Apparently in consequence of omitting to verify their diagnoses by isolating and testing doubtful organisms very divergent views are held even at the present day by various observers as to which of the various types of organisms should be considered dangerous. This unsatisfactory confusion renders many of the investigations untrustworthy, and can only lead to the discredit of the bacteriological diagnosis of diphtheria.

The divergent views held on this question are well illustrated by the Report of the Massachusetts Committee on "Diphtheria bacilli in well persons" (1902).

The several collaborators were requested to detail the various bacilli according to Wesbrook's types (1900), and also to state in each case whether or not a positive diagnosis of the presence of diphtheria bacilli had been made.

In Providence (Footnote, Report, p. 24) on the basis of the Committee's belief that *A*, *C* and *D* of Wesbrook's types should be considered chiefly, or solely, important, there would be 43 % of positives (*i.e.* cultures in which diphtheria bacilli were present). If all granular, or barred forms, but not the solid forms, be included as Prof. Gorham of Providence states, there would be 3 % of positives. If all be included there would be 25 %. The number actually reported positive (*i.e.* diphtheria bacilli present) makes about 9 %.

In Washington the positives formed 9 % on the Committee's standard; but 22 % were reported positive. In Boston on the Committee's standard 3.02 % were positive, but only 1 % were so reported.

It is evident from the above statement that some standard must be adopted in dealing with an epidemic. In order to prove the reliability, or otherwise, of the standard adopted, although it involved a considerable increase of work, I have isolated, cultivated on several media, and tested on animals 113 out of the 117 diphtheria bacilli discovered as well as several organisms derived from various sources resembling in morphology the diphtheria bacillus¹.

Hofmann's pseudo-diphtheria bacillus was also very frequently found and numerous pure cultures were made. The observations on this point are given later (page 297).

Morphology of the diphtheria bacillus.

Cobbett (IV. 01) has carefully worked out the virulence of the various types of bacilli met with during the outbreak of 1900 at

¹ For results see table at the end and descriptions of diphtheria-like organisms.

Cambridge. A standard based on these observations was adopted by him in the outbreaks of 1900 and 1901 at Cambridge and by myself at Colchester (1902) and in this epidemic.

He recognizes five morphological types of diphtheria bacilli on young serum cultures.

- (1) Oval bacilli with one unstained septum. Very young forms¹.
- (2) Long, faintly stained, irregularly beaded bacilli (Plate XIV, Fig. 1).
- (3) Long regularly beaded bacilli—"streptococcal" forms (Plate XIV, Fig. 2).
- (4) Segmented bacilli (Plate XIV, Figs. 3, 4, 5).
- (5) Uniformly stained bacilli (Plate XIV, Fig. 6).

In all these types except the first the organisms are usually considerably, occasionally three or four times, longer ($3-6\ \mu$) than the pseudo-diphtheria bacillus. They are nearly always decidedly curved, of varying thickness, and often with decidedly clubbed ends. The arrangement of the bacilli in the field has been likened to Chinese characters, or pine needles on the ground.

The medium used throughout this and the other epidemics just mentioned was clear alkaline ox, or horse serum to which 1% of glucose had been added, first prepared by Prof. Lorrain Smith of Belfast (1894).

The morphological appearances were those noted after 18—24 hours' growth on this medium at 37° C.

Several experiments were made to ascertain whether different alkalis (NaOH, KOH and Na₂CO₃) made any difference in the morphology, but none was noted. For routine purposes the alkali used was a 10% solution of caustic soda, and it was generally found necessary to add about 1 c.c. to every 100 c.c. of the serum.

This medium has the advantage of being clear so that the differences of the colonies can be easily seen, also diphtheria and Hofmann's bacilli grow readily in 24 hours to the exclusion of most other organisms.

Comparative experiments between Löffler's medium and the above have convinced me that both organisms grow as readily on the latter as on the former, and that the morphology and cultural characteristics of both organisms are the same on these media. On the clear alkaline serum the inspection of the colonies is more readily accomplished, and

¹ The author does not mean to imply that in any pure culture these would be at any time the only forms met with. Certainly in my experience this has never been the case.

consequently it is easier to pick out suitable colonies for examination and isolation at an early stage. In this respect the alkaline serum is superior to Löffler's, and since in all other respects it is equally good, it has been used throughout for diagnostic work.

On one occasion ox serum could not be obtained and human pleuritic exudate was made use of. The result was that the Hofmann's bacillus became difficult to differentiate from the diphtheria bacillus, owing to large numbers of segmented forms being observed. In consequence a considerable number had to be subcultivated before a certain diagnosis could be given, but after subcultivation only typical Hofmann's bacilli were found.

Subsequent experiments with media made from human serum and pleuritic exudate showed that in every instance diphtheria bacilli became longer, more curved and segmented on these media as compared with the same organisms on alkalized ox, or Löffler's serum. The Hofmann's bacillus on human serum showed a tendency to become longer and develop segmentation, and on human pleuritic exudate was often definitely segmented, and curved. In fact many of the organisms closely resembled those shown in Plate XV, Fig. 7.

Media made from hydrocele fluid as recommended by White (1895) and Hayward (1895) were unsatisfactory.

The colonies of diphtheria and pseudo-diphtheria bacilli on alkalized serum after 24 hours' growth at 37° C. are the same in size and appearance, namely small, round, smooth, gray, and dome-shaped.

Methods of obtaining cultures and microscopical examination.

Swabs were prepared, constructed of cotton-wool wrapped round a short wire. These were placed inside stout glass test-tubes and sterilized.

In obtaining a culture for examination the throat or nose of the person was wiped with the swab, which was then returned to its case. As soon as possible the infected swab was rubbed on the surface of a serum tube. The culture so obtained was grown at 37° C. for 18—24 hours.

At the time of the examination samples from dissimilar colonies were streaked on cover-glasses by means of a sterile platinum needle, stained with diluted Löffler's methylene blue, mounted in the stain

and examined under $\frac{1}{12}$ oil immersion lens¹. Unless the growth was very scanty, or the culture very thickly studded with colonies, organisms from more than one colony were never placed on the same position on the cover-glass. By this means the appearance of the various colonies, and the morphology of the bacilli derived from them could be studied, and the difficulties of distinguishing the organisms in a general smear avoided (see p. 316).

This method has very considerable advantages over the method generally adopted of staining with Löffler's methylene blue and finally mounting in Canada balsam. In the first place much time is saved, the bacilli are not overstained, nor are they liable to be distorted during manipulations or by the action of the balsam. Comparative experiments by the two methods showed that in balsam the diphtheria bacilli and others appeared smaller, and shrunken, and were much less easily distinguishable from other organisms than when treated by the method just described. Finally this procedure allows of 5% acetic acid being run under the cover-glass as recommended by Cobbett (ix. 1901). This results in the organisms being partially decolourized, but the polar bodies in the case of diphtheria and other bacilli possessing them remain stained, and stand out as black dots in the faintly stained bacilli. A single group of doubtful organisms can thus be readily examined for the presence of polar bodies without removing the eye from the microscope, a matter of great importance when the organisms are few, and it is doubtful whether they can again be obtained for making a preparation by Neisser's method.

Comparative experiments with this method and that described by

¹ More in detail the process consists of making on a cover-glass several parallel streaks from different colonies. To accomplish this the top of a colony is touched with a platinum needle, the organisms separated by rubbing the point in water, and then drawing the needle across the cover-glass. By this means thin bands of well separated organisms from various colonies are left on the cover-glass. The cover-glass is allowed to dry and then dropped film side down on a glass slide on which has been placed a drop of dilute Löffler's methylene blue (1:5). The specimen is almost immediately firmly pressed down on filter-paper with the cover-glass downwards, with the result that the excess of stain is forced from under the cover-glass, and absorbed by the blotting-paper. Immersion oil is then placed on the cover-glass and the preparation examined mounted in a small quantity of the staining fluid. If the films are properly prepared no bubbles are found under the cover-glass, and the organisms take up the stain and appear to lie in a clear fluid (see Cobbett and Phillips, xii. 1896, p. 197). If segmented the segments are shown as dark blue bands and the presence of polar bodies is generally indicated.

Neisser show that the results are identical. By the adoption of these procedures the time required in making examinations is much shortened, and with experience over 100 cultures can be thoroughly examined by one observer in the course of the day.

Polar bodies.

Contrary to the experience of most observers a large number of the diphtheria bacilli found during this epidemic possessed in the original cultures few and small polar bodies or none. This was especially noted in the bacilli obtained from both notified persons and contacts infected with virulent bacilli from the St Matthew's School in both outbreaks and the Sturton Street School.

Most of these bacilli were of the type shown on Plate XIV, Fig. 3. This condition was not so common amongst the later cases when well-marked polar bodies were usually present.

Those bacilli which were originally without polar bodies usually did not show them in the first subcultures, but after continued growth on artificial media they made their appearance, and were present in fresh subcultures after 18 hours' growth.

The lack of polar bodies has been taken by some authorities to indicate a want of virulence, but in this epidemic polar bodies were more often absent in the virulent than in the non-virulent forms.

Of the 88 virulent diphtheria bacilli discovered 43.1 % showed well-marked, 30.7 % small and poor, and 26.1 % no polar bodies; and of the 25 non-virulent diphtheria bacilli 88 % showed well-marked, 8 % small, and 4 % no polar bodies.

Cultures of these organisms grown on Löffler's serum behaved in the same way.

Hofmann's pseudo-diphtheria bacillus had as a general rule in young cultures no polar bodies, but in a few instances inconspicuous polar bodies very few in number were seen. In subcultures from colonies of the latter the organisms were of the usual type without any signs of polar bodies. In older cultures small and indistinct polar bodies occurred in a small proportion of the organisms.

Most of the diphtheria-like organisms (pp. 301—312) possessed very distinct polar bodies.

The presence of polar bodies is therefore a considerable aid to diagnosis, but their absence in organisms morphologically resembling diphtheria bacilli does not prove that they are not virulent and dangerous diphtheria bacilli.

Cultural characters of the diphtheria bacillus.

All the diphtheria bacilli that were discovered in, and isolated from, 113 different persons were grown in glucose broth. All without exception formed acid in 48 hours, whereas no single specimen of the large number of Hofmann's pseudo-diphtheria bacilli which were grown on this medium did so.

The growth of the diphtheria bacilli in sugar-free, or glucose broth was in all instances as generally described, namely the fluid remained clear and a deposit of fine granules formed on the bottom and sides of the test-tube. The deposit was generally more marked in cultures in glucose broth.

This test for the production of acid, which, according to most observers, ranks next in importance to the inoculation of animals, although it serves to differentiate the diphtheria bacillus from the pseudo-diphtheria bacillus, does not distinguish the former from certain other diphtheria-like organisms (pp. 301—312).

All the diphtheria bacilli were also grown on slightly alkaline potato, and in most cases there was either no visible growth, or a very slight white glazed film. Certain of the diphtheria-like organisms however grew luxuriantly on this medium.

Numerous cultures on *gelatin* and *agar slopes* failed to show any constant well-marked differences between diphtheria and Hofmann's bacilli. It was found, however, that in *agar stab* cultures the diphtheria bacillus frequently only produced a scanty and thin growth on the surface, though in some instances it was fairly abundant. Hofmann's bacillus on the other hand grew extremely well on the surface, producing a white, rounded, smooth, luxurious mass occasionally marked by concentric rings.

In the examination of certain ear discharges and normal ears (p. 311) organisms resembling the diphtheria bacillus were found which grew well on potato, and it occurred to me that a differential medium might be made from potato juice stiffened by the addition of agar. This *potato-agar* medium was made, and it was found that diphtheria bacilli produced characteristic colonies, entirely different from those of any other diphtheroid organisms which have been tested.

This medium was prepared in the following manner. The pulp and fluid obtained by crushing washed, peeled, potatoes in a mincing machine was added to water in the proportion of one gramme of pulp to 1 c.c. of water. The mixture was allowed to stand in a flask for 12 hours and then was filtered through filter

paper. Agar after the usual treatment, to the extent of 3 %, was then added to the filtrate, and the flask was placed in a steam sterilizer at 100° C. till the agar was melted. After cooling the white of egg was added, and the flask again placed in the steam sterilizer till the fluid was clear. The medium was then filtered through a Chardin filter paper, filled into tubes, and sterilized on three occasions at 100° C. in steam. This is spoken of as *Acid Potato Agar*.

After standing, the mixture of pulp and water is decidedly acid. A second medium called *Alkaline Potato Agar* was made by neutralizing the acid with normal caustic soda, using litmus as the indicator, and adding 3 c.c. of alkali per litre before the addition of the agar.

The first of these media is clear, but slightly opalescent like ordinary agar, and the second is also clear but of a brownish colour.

As it occasionally happens that on tubes from one flask of a medium an organism produces a certain type of colony, but on tubes from other batches of the medium, though made in the same way, another type grows, both kinds of the potato-agar medium were made on four separate occasions at considerable intervals. Diphtheria and Hofmann's bacilli and most of the other organisms described later behaved in the same manner in all the samples of this medium whether acid or alkaline. Only two organisms differed markedly in their growth in the two varieties of this medium.

Although subsequent experiments may show that this medium is not so reliable as I have found it to be, yet it seems worthy of an extended trial. During this outbreak nearly all the 113 diphtheria bacilli, which have been isolated, as well as all the diphtheroid organisms (each several times) and nearly 100 pure cultures of Hofmann's bacillus have been grown on it, and no difficulty has yet arisen in differentiating by their growth after 48 hours the diphtheria bacilli from all the other organisms.

The colonies of the diphtheria bacillus on acid, or alkaline potato-agar are small and transparent, or gray, after 24 hours' growth at 37° C. After 48 hours, however, they have considerably increased in size. Two somewhat different forms occur.

(a) One is flat, gray, and round, with a small more opaque slightly raised mass in the centre, which has a rough granular surface. The rest of the colony has also a slightly granular surface, but the granulations are much finer (Plate XVII, Fig. 1). Very rarely such colonies have deep indentations and are star-shaped (Plate XVII, Fig. 2).

(b) The second type of colony is also round and flat, but is almost transparent. Again there is a small elevation in the centre, which in this case is very slightly granular, surrounded by an almost transparent

faintly granular zone, but the edge of the colony is raised in a decided rim as high as the central elevation (Plate XVII, Figs. 3 and 4).

Both these types of colony are more pronounced when the colonies are sparsely scattered. When the colonies are closely aggregated they never attain a size sufficient to show their characteristic features.

These two types of colony do not seem to be in any way connected with the virulence or want of it in the organisms. The great majority formed the type (*a*), and frequently these as the results of longer growth came to closely resemble type (*b*).

Hofmann's pseudo-diphtheria bacillus forms medium-sized, round, whitish, opaque, smooth, dome-shaped colonies entirely different from the above (like the colonies shown on Plate XVII, Fig. 5). All the other diphtheria-like organisms investigated with two exceptions form also colonies more or less of the dome-shaped type.

Consequently this medium, in one or other of its forms, seems to provide an additional and ready means of separating the diphtheria bacillus from other bacilli which resemble it in morphology, and Hofmann's bacillus.

Several specimens of the diphtheria and Hofmann's bacillus were tested for the formation of indol, but in most cases none was formed, or the quantity produced was very small, whereas some of the diphtheria-like organisms produced it in abundance.

According to Hewlett (1901) in the case of the diphtheria and of the Hofmann's bacillus the pink reaction obtained by the addition of strong acid to the weak nitrate solution is not due to indol, which is volatile, whereas this is due to a non-volatile substance, skatol-carbolyx acid.

The virulence of the diphtheria bacillus.

The great majority of those who have investigated this subject have come to the conclusion that diphtheria bacilli show all degrees of virulence, from the highly virulent bacilli which in doses of .1 c.c. of a 48 hours' broth culture injected subcutaneously kill half-grown guinea-pigs within three days to organisms completely lacking in virulence of which 2 c.c. produce no effect.

Abbott (1902, p. 400), for example, says, "under certain circumstances with which we are not acquainted *Bacillus diphtheriae* may become diminished in virulence, or may lose it entirely, so that it is no longer

capable of producing death of susceptible animals, and may cause only a transitory local reaction from which the animal entirely recovers.

"This exhibition of the extremes of its pathogenic properties, viz., death of the animal, on the one hand, and only very slight local effects on the other, was at one time thought to indicate the existence of two separate and distinct organisms that were alike in cultural and morphological peculiarities, but which differed in their disease-producing power. Further studies on this point have, however, shown that the genuine diphtheria bacillus may possess almost all grades of virulence."

In fact the prevailing opinion appears to be that under certain unknown conditions, or from lengthened stay in the throat, the virulent diphtheria bacillus gradually becomes attenuated until it may become completely non-virulent.

This opinion has been arrived at by the discovery of diphtheria bacilli, which only kill guinea-pigs after several days, and others which only cause local tumour without death. Councilman (1893, p. 548) for instance thinks that "like other organisms the diphtheria bacillus varies greatly in virulence," and Andrews (1900) considers that "short of fatal results the production of pathogenic effects, such as illness and local tumour in the inoculated animal is consonant with the belief that the bacillus is an attenuated form of the diphtheria bacillus."

At least two observers, however, have found that in their experience there are no intermediate degrees of virulence between the fully virulent and completely non-virulent organisms.

Theobald Smith and Walker (1896) in a series of most carefully conducted experiments on the toxin-producing power of 42 cultures of diphtheria bacilli from different sources found that all the organisms produced the same amount of toxin, or were of equal virulence. Eleven of these cultures were made from the throat 15—62 days after the disappearance of the disease. They did not meet with any non-virulent forms.

Spronck (1895, 1898) showed that the variations of toxin-producing power of diphtheria bacilli grown in broth were due to the presence of varying quantities of muscle sugar in the medium, and recommended a sugar-free broth for the production of toxin.

Theobald Smith (1896) also pointed out "that the amount of toxin decreased as the presence of muscle sugar grew larger, and that the best was obtainable only from beef nearly free from this substance."

In the majority of observations on the virulence of diphtheria bacilli no mention is made of the kind of broth in which the organisms

were grown, or the dose injected, and it is possible that the condition of the broth as regards muscle sugar may to some extent account for the various degrees of virulence which have been met with.

More recently Cobbett (x. 01, p. 496) says: "I do not deny that diphtheria bacilli may become attenuated, but think it interesting to note that in a somewhat extended experience partially attenuated bacilli have never been found. Fifty-five diphtheria cultures have been separated and tested for virulence during the spring, making with the 24 isolated and tested during the autumn and winter 79 in all." Moreover in seven cases he tested the virulence of the bacilli present on from two to ten occasions and found it constant.

My experience during this epidemic is in entire agreement with that of these authors. 113 cultures of organisms morphologically and culturally identical with diphtheria bacilli have been isolated and tested with the result that 87 have been found capable of killing half-grown guinea-pigs in three days, and 25 have been completely non-virulent. One for reasons to be given later, only killed in 12 days.

In order to render the conditions in these experiments on virulence as far as possible constant, almost all the bacilli were grown for 48 hours at 37° C. in muscle-sugar-free broth from the same stock. It was found that all did not grow in this medium equally well on the first inoculation. In cases in which the growth was poor a second tube was inoculated by transferring a loopful of broth from the first tube to a second. Under these conditions, when the organisms became accustomed to the medium, abundant growth was generally obtained.

Guinea-pigs weighing between 150 and 400 grammes were selected, and from .1 to .3 c.c. of the 48 hours' broth cultures was injected subcutaneously.

Under these circumstances, when the conditions had been made as constant as possible, 87 specimens of diphtheria bacilli killed within three days, or at the outside limit on the fourth day. Leaving some margin for the differences due to the inequalities of growth, the susceptibilities of the animals and their different sizes, all of these may be regarded as fully virulent bacilli.

The one specimen which only killed after 12 days could not be induced to grow well in broth, only a few granules resulting after 48 hours' incubation. Several transferences from one broth culture to another still resulted in very poor growth. Consequently the dose was much smaller than in the other cases, and it cannot be assumed that this was an attenuated form.

Cobbett (x. 01, p. 496) also, in two instances, found organisms which grew poorly in broth, and only killed after several days, one on the 11th and the other on the 7th day. In each case, however, when the injections were repeated with a new culture death took place in the usual time.

Autopsies were made on all the animals which died. In every case the well-known signs of death from experimental diphtheria were found, namely subcutaneous oedema, general congestion of the organs, especially marked in the supra-renal capsules, and in some cases fluid in the pleura.

At the site of injection a small grayish mass was always found from which the bacilli could be obtained. In all cases cover-glass preparations were made from this point, and the morphology of the bacilli noted. All the various morphological types of the bacilli, which have already been described as occurring in cultures, were found in these preparations¹. The gelatinous oedema varied greatly in amount; from a comparatively small area round the site of injection to a condition in which the whole ventral subcutaneous tissue of the abdomen, thorax and thighs was involved. In most cases this oedematous mass was clear and gelatinous, but in a certain proportion of cases minute haemorrhages had occurred into its substance. The conditions have been described in the tables at the end as extensive, moderate, slight and haemorrhagic.

The degree of injection of the supra-renal capsules was also variable, ranging from dark red, almost black, to pink. The conditions met with have been described by the terms dark red, red, and pink in the tables.

In these autopsies the condition of the lungs was especially noted. They varied from deep red, mottled with darker areas, to pink or whitish.

As far as possible the quantity of fluid in the pleural cavities was ascertained. This varied from 12 c.c. to none. The fluid in all cases was clear, without any trace of blood, and no relationship seemed to exist between the quantity of fluid and the degree of injection of the lungs.

In most of the earlier experiments '1 c.c. of a 48 hours' sugar-free broth culture was injected, but in some of the more recent ones up to '3 c.c. was used. This was done in order to ascertain whether the larger doses within these limits caused death to take place earlier or produced more extensive lesions. The average time of death was, however, not altered, and no differences were discovered at the autopsy.

The injection of these small doses ('1—'3 c.c.) in the case of non-virulent

¹ Compare Ohlmacher, p. 290.

bacilli produced no results, or only a very transitory oedema lasting a few hours. In many of these cases other animals were injected with doses of 2 c.c. with the same results. Roux and Yersin (1890, p. 47) have pointed out that in some cultures they obtained colonies some of which were virulent and others non-virulent. To guard against the possibility of having separated non-virulent bacilli only from patients in whom virulent bacilli were also present, the second injection, whenever possible was made with bacilli isolated from another culture. Also the organisms were taken from several colonies in this culture. With these precautions against missing any virulent, or slightly virulent, bacilli, the results were always the same, viz. the inoculation of 2·0 c.c. gave rise to no tumour or signs of illness in the inoculated animal.

It will be noticed that in several (10) of the inoculation experiments given at the end of the table (p. 327) 2·0 c.c. of broth culture were injected on the first occasion. The cultures were derived from persons who were known to have been in contact with patients in whom non-virulent bacilli occurred. In every instance the bacilli were found to be non-virulent, though identical with diphtheria bacilli in other respects.

The larger dose was also used in the case of cultures derived from persons who were suffering from very mild sore throats, and in whom inquiry revealed no possible source of contact with persons harbouring virulent bacilli. In all these cases with one exception (116) the organisms were non-virulent.

So far as I am aware this is the only outbreak of any size in which the diphtheria bacilli, from almost every person found to harbour them, have been isolated and tested. Moreover in no other outbreak on so large a scale have the persons liable to be infected been so thoroughly investigated.

On glancing over the table it will immediately be seen that the proportion of non-virulent bacilli bears no relation to the number of virulent bacilli either in notified persons or contacts. Moreover in the first three schools in which large numbers of children were examined the percentages of non-virulent bacilli were alike (·9%), whereas the percentage of virulent bacilli varied widely in both notified cases (4·3—6%) and infected contacts (4·1—1·6%). In the other institutions small numbers of persons only were examined in each case, consequently the percentages obtained are not of much value, but when the proportions are taken on the total number of persons examined in the last nine, it is found that the percentage of non-virulent bacilli is nearly the same as in the first three schools.

The table gives the proportion of persons in, or connected with, infected, or non-infected, institutions in whom virulent, or non-virulent, diphtheria bacilli have been found.

The total percentages of the bacilli obtained from notified cases and contacts with virulent bacilli was 6.4% and with non-virulent bacilli 1.8%.

+ These include the persons just enumerated together with certain other notified persons and family contacts. Four persons first discovered as contacts and subsequently notified have been placed under the head of "Notified" in the totals only.

The records of two persons with virulent bacilli have been omitted by mistake from the tables at the end. They are recorded below. The significance of the symbols may be seen by reference to the tables at the end. Both at the time of writing were still isolated.

116	W.F.	(a)	A. Δ .A. Δ .A. Δ .O.A.O. Δ .	*	* *	30	acid	245	2·0	MS +	extensive	dark red	1·5 c.c.
117	A.S.	(a)	A. Δ .			?	" "	300	·2	SS LS +	" "	-	-

+ One person in each case added in whom the virulence not tested, but assumed in the one instance to be virulent and in the other non-virulent, as this was the case in two and four other members of their families tested (94, 109).

The proportion of non-virulent diphtheria bacilli in the whole number of persons examined was 1·8%.

It is therefore evident that in this epidemic at any rate from 1 to 2 persons in every hundred, whatever the proportion infected with virulent bacilli, harboured non-virulent diphtheria bacilli in their throats. There is a remarkable coincidence between these figures and those obtained by the examination of 1500 normal persons (p. 262) amongst whom about the same number (2·05 %) showed non-virulent bacilli.

In three households five (107—111), four (96—99) and two (74 and 82) persons had non-virulent bacilli. It may be safely assumed that the remaining members were in each case infected from one person. If these eight persons be excluded there remain 18 (1·2%) in 1401 who harboured non-virulent bacilli not derived so far as is known from other persons possessing them.

Two instances occurred in which one member of a family possessed virulent bacilli and a second member non-virulent, but in each case the member harbouring the virulent organisms had recently been in close contact with other persons in a similar condition.

Consequently in this epidemic there is no evidence to show that non-virulent diphtheria bacilli can give rise to virulent, but on the other hand the three families just mentioned demonstrate that non-virulent bacilli when transferred from one person to another still remain non-virulent. Moreover no clinical cases of diphtheria are known to have arisen from such persons although no less than 13 of them refused to be removed to the Isolation Home. It is also worthy of note that none of the notified cases from which non-virulent diphtheria bacilli were isolated showed any marked clinical signs. In fact without the discovery of the organisms the illness would in most cases have been passed over as a slight sore throat. In the cultures derived from the majority of these cases colonies of the diphtheria bacillus were rare, but colonies of other organisms, especially cocci, were abundant. It would seem likely therefore that the clinical symptoms were entirely due to the latter.

All these observations tend to show that diphtheria bacilli which are non-virulent for guinea-pigs are also non-pathogenic to man. If this conclusion be correct it may be questioned whether it is necessary to isolate these persons, and whether, if isolated amongst those who carry about the virulent diphtheria bacillus, they are not liable to catch diphtheria. In practice, however, the virulence of the bacillus can only be determined after isolation has been carried out, and

accordingly several children with non-virulent bacilli lived in the Isolation Home with others who had virulent bacilli. This, however, was followed by no bad results.

It should be noted in this connection that Cobbett (x. 1901, p. 491) found that those admitted to the Home with a non-virulent bacillus were never found to have acquired a virulent bacillus during their stay, nor was a non-virulent bacillus ever found in a child in whom virulent bacilli had once been found. In the case of one child the bacilli were isolated and tested 10 times in the course of 15 weeks and were always found to be non-virulent. During five weeks her sister was with her constantly, and on three occasions bacilli were isolated from her and found fully virulent. Moreover from another girl, who remained in the Home about as long, diphtheria bacilli were isolated and found fully virulent on no less than six occasions.

There is no evidence to prove that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli, or originate diphtheria. Shattock (1898) experimented on this question and found that it was impossible to raise the virulence of lowly virulent diphtheria bacilli by cultivating them in a current of sewer air, even after two months.

One cannot deny that the virulent diphtheria bacillus may become attenuated, *but it is interesting to note that in the two most extended observations on this subject, namely the two consecutive outbreaks investigated by Cobbett and this one, in which 79 and 113 specimens of diphtheria bacilli, 192 in all, have been isolated and tested for virulence, no partially attenuated diphtheria bacilli have been found.*

Taking into consideration the morphological and cultural resemblance of the Xerosis bacillus, frequently present in the normal eye, to the non-virulent diphtheria bacillus, the very close resemblance of certain organisms found in the mouth and ear, and the similar distribution of non-virulent diphtheria bacilli in contacts and non-contacts, it may be that the older view which regarded many of the latter as belonging to a distinct species may be more correct than the one at present generally accepted.

As will be shown later (pp. 301—312) some of the organisms obtained from the mouth and ear so closely resemble non-virulent diphtheria bacilli that they would be by many observers undoubtedly classed as such.

The persistence of diphtheria bacilli in the throat.

In several of the recorded outbreaks patients and infected contacts have been released from isolation after one negative examination. The need for more than one negative examination has, however, been clearly established. Hill (1898) states that the Boston Board of Health, U.S.A., require two consecutive negatives from convalescents, and three from hospital patients before they are declared free from infection.

At the South Western Fever Hospital, London, the patient is detained until the bacilli disappear as evidenced by three consecutive negative examinations. Cobbett (iv. 1901, ix. 1901) requested the practitioners to submit swabs till three consecutive negative examinations were obtained. He found on more than one occasion that two consecutive negative examinations were followed by the discovery of bacilli.

In this outbreak the mean duration¹ in the throat of virulent diphtheria bacilli amongst notified persons in whom the disease was not fatal was 36 days, of non-virulent bacilli 15 days. Amongst contacts with virulent bacilli it was 30 days, and with non-virulent bacilli 25 days. The length of their stay in notified persons with virulent bacilli varied between 99 and 8 days, in notified persons with non-virulent bacilli between 51 and 1 days, in healthy contacts with virulent bacilli between 57 and 8 days, and in contacts with non-virulent bacilli between 122 and 8 days.

The following table gives the results of some of the investigations on this subject.

TABLE V.

Observer	Mean duration of persistence of diphtheria bacilli in the mouth	Longest period noted	Shortest period noted	
Park (1894)	8	49	3	(from the disappearance of the exudate)
Morse	10	—	—	
Bissel (v. 1902)	14	—	—	
Cobbett (iv. 1901)	18	49	3	(3 consecutive negatives required)
„ (x. 1901)	—	108	—	
Massachusetts Board of Health (1901)	27	185	2	(for a period of 5 years)
Graham-Smith (1902)	28	94	—	(3 consecutive negatives required)
Woodhead (1896)	52	200	—	
Wesbrook (1900)	—	135	—	

Most of these observers require less than three consecutive negative examinations for release.

¹ The duration of persistence is reckoned from the date on which the bacilli were first found to the date of the first of the three consecutive negative examinations.

Out of the 104 convalescent patients carefully examined at Colchester (Graham-Smith, iv. 1902) on many occasions prior to discharge, one negative followed by the finding of diphtheria bacilli occurred in 11, two consecutive negatives in 10, three consecutive negatives in one, and four in one. Amongst 45 healthy infected contacts on eleven occasions diphtheria bacilli were again encountered after one negative examination, on three after two consecutive negatives and once after four.

In this outbreak a single negative followed by the reappearance of the diphtheria bacilli often for a long period occurred on 49 occasions, two consecutive negatives on 22 occasions, three on two occasions, and six on one.

These misleading negatives may be due to the faulty taking of swabs, swabbing too soon after the application of an antiseptic, or to the bacilli lurking in the crypts of the tonsils or sinuses connected with the nasal cavities, and finding their way thence into the pharynx.

Wolff (1895) found diphtheria bacilli in fatal cases in the frontal and ethmoidal sinuses, and the antrum, and Councilman, Mallory and Pearce (1901) in the antrum and middle ear.

A glance at the tables and the figures just given shows that two consecutive negatives, and in some cases even three, are not a complete safeguard. In practice, however, it is occasionally difficult to enforce isolation till three consecutive negatives¹ have been obtained, and to insist on more would be impossible. Therefore, I think, whenever practicable, a minimum of three consecutive negatives should be enforced before convalescent cases, or infected contacts, are freed from isolation.

Hofmann's pseudo-diphtheria bacillus.

The pseudo-diphtheria, or Hofmann's, bacillus is an organism very frequently met with in the throats and noses of the scholars at the public schools.

Much diversity of opinion has existed and to some extent still exists, as to the relationship between this organism and the diphtheria bacillus. On the one hand there are some who consider that it is merely an attenuated form of the true diphtheria bacillus, capable under favourable conditions of developing into the latter, and giving rise to diphtheria, others appear to think that only certain species included under this

¹ See observations on this point p. 271.

name are attenuated diphtheria bacilli, whilst others again consider that this organism is in no way related to the diphtheria bacillus, and is at all times perfectly innocuous to man.

The question of the relationship, if any, which exists between these two bacilli is not a matter of scientific interest alone, for the entire management of an outbreak from the bacteriological standpoint depends on the view which is taken. Hewlett (1899, p. 203) for example, believing "that Hofmann's pseudo-diphtheria bacillus is a modified diphtheria bacillus," goes so far as to say that a positive diagnosis of diphtheria should be given whenever it is found.

If the opinion of those who consider Hofmann's bacillus to be merely an attenuated variety of the diphtheria bacillus be correct, measures such as have been described, and which in several outbreaks have proved so efficacious, ought to be of little use, since (according to this view) organisms capable under certain unknown conditions of giving rise to diphtheria remain in the throats and noses of nearly half the school children. Had this view been adopted, about 500 school children in Cambridge would have had to be isolated in order to render the measures efficacious.

These observers base their opinions on certain experiments, and on the statement that the pseudo-diphtheria bacillus is found more frequently amongst convalescents from diphtheria, and persons who have been in contact with cases of the disease, than amongst normal persons.

Some of the experimental evidence in favour of this view is as follows:

Roux and Yersin (1890, p. 418) were able to attenuate virulent diphtheria bacilli by growing them at a high temperature. Although they were able to increase the virulence of lowly virulent bacilli, they were unable to do so with completely non-virulent forms (p. 423).

Hewlett and Knight (1897) considered that on one or two occasions they succeeded in transforming a pseudo-diphtheria into a virulent form, but have been apparently unable to repeat these transformations. Richmond and Salter (1898) briefly stated that by repeated passages through certain birds they had been able to convert Hofmann's into diphtheria bacilli virulent for guinea-pigs, and Salter (1899) in the following year gave details of some of these experiments.

Ohlmacher (1902) experimented with three organisms, and concluded that by a short sojourn in an immune animal a diphtheria bacillus may be converted into a pseudo-diphtheria, and that the reverse may be

brought about by passing the organism through a susceptible animal. His experiments only show, however, that a long granular diphtheria bacillus, after recovery from the subcutaneous tissues of a rat, became short and uniformly staining, but still formed acid in glucose media. A uniformly staining, but pathogenic, bacillus after recovery from the spleen of a guinea-pig became granular, and a short uniformly staining and slightly virulent bacillus (killing in 7 days) after passage through an immune animal became granular and non-virulent.

Lesieur (1901) in agglutination experiments on the two organisms concluded that certain species of the pseudo, but not others, were identical with diphtheria bacilli. On the other hand Lubowski, working with the serum of animals immunized to a non-virulent diphtheria bacillus, found that it agglutinated not only these bacilli, but 23 quite typical races of diphtheria bacilli. The serum had no action on pseudo-diphtheria bacilli.

The above are some of the main experimental arguments in favour of the identity of the pseudo-diphtheria and diphtheria bacillus, "but before accepting the conclusion that *B. Hofmanni* is convertible into *B. diphtheriae*, and on this account a factor in the causation and spread of diphtheria, more evidence is necessary, particularly with regard to the strict purity of the cultures used, and also with regard to the number of cases in which such a conversion may be considered to have occurred. Caution is particularly necessary when it is called to mind that the evidence at one time advanced in support of the conversion of *B. anthracis* into *B. subtilis*, and at another of *B. coli communis* into *B. typhosus*, has in both cases been discredited" (Gordon, 1901, p. 420).

In support of the view that the pseudo-bacillus is an attenuated form of the diphtheria bacillus, it has been repeatedly stated that it occurs more frequently in the throats of those who are recovering from an attack of diphtheria at the time when the true diphtheria bacillus is disappearing. It must be remembered, however, that in diagnostic work, when once the diphtheria bacillus has been found no further search is usually made, but that in subsequent examinations, when the diphtheria bacilli are becoming less numerous, a more careful examination is necessary, and the pseudo-bacillus is found and recorded.

During this epidemic Hofmann's pseudo-diphtheria bacillus has been considered to be entirely unrelated to the diphtheria bacillus and innocuous to man, but though no importance has been attached to its presence it has been recorded whenever it has been found.

Twenty-three cultures of this bacillus derived from persons suffering

from diphtheria, or infected with diphtheria bacilli, were isolated and tested on animals to ascertain whether they showed any signs of virulence. Six of these were isolated from the first cultures, and three from the second from notified persons (all with virulent diphtheria bacilli), eight from infected contacts at the first examination (seven with virulent and one with non-virulent diphtheria bacilli), and seven from contacts at the second examination (seven with virulent and one with non-virulent diphtheria bacilli)¹. If this organism is an attenuated form of the diphtheria bacillus it would seem probable that under these circumstances, when the true diphtheria bacillus was still present in very large numbers, its virulence would not have been completely lost. *All, however, behaved in the manner typical of the pseudo-diphtheria bacillus, giving an alkaline reaction in glucose broth, and being completely devoid of virulence for guinea-pigs in doses of 2 c.c. of 48 hours' broth cultures.*

Further, it will be seen by reference to Table VIII, p. 297, and to the more detailed tables at the end of the paper, that Hofmann's bacillus was not found more frequently in the throats of convalescents and healthy infected contacts than in those of normal persons, although the former were all examined on many occasions. In most cases it was either present or absent throughout the series of cultures examined from each person, and did not appear to be in any way related to the diphtheria bacillus.

The characters of the pseudo-diphtheria, or Hofmann's, bacillus.

The organism which in these observations is called the pseudo-diphtheria, or Hofmann's, bacillus has the following characteristics.

In young serum cultures it appears as a darkly staining oval bacillus of somewhat variable length, with one narrow unstained septum. Occasionally colonies are met with, which contain a fair number of bacilli with several septa. Young subcultures from these colonies, however, usually show only the typical oval forms. It frequently happens also that besides the well-stained forms oval unstained specimens of the same size and shape are found. There is a marked tendency for these organisms to be arranged in small groups, several members of which lie

¹ Notified persons from whom subcultures of Hofmann's bacillus were obtained at the first examination were 11, 12, 24, 39, 54, 88, at the second examination 33, 100, 116. Infected contacts were 7, 8, 22, 32, 65, 75, 81, 99 at the first, and 4, 6, 16, 17, 23, 25, 79 at the second examination.

parallel to one another. No polar bodies usually occur in young cultures, though occasionally colonies are found in which some of the organisms have very small indistinct polar bodies.

Besides these typical oval forms, I have, both on previous occasions and in this epidemic, met with colonies of segmented bacilli in which very few, if any, of the small typical Hofmann forms occurred. These organisms are clubbed, but broader and take the stain more deeply than diphtheria bacilli. The stained segments are very dark, and the septa narrow and well-defined, running in all cases transversely across the bacillus. They do not show any polar bodies by Neisser's method, produce an alkaline reaction in glucose broth, and are non-pathogenic to guinea-pigs. Moreover in subculture they revert to the typical short form of Hofmann's bacillus with an occasional long specimen. It is only after several days' growth in subculture that many long segmented forms become visible. This is termed the pseudo-diphtheria type of Hofmann's bacillus (Plate XV, Figs. 7 and 8)¹. This type has generally been found in the noses of persons suffering from catarrh, and occasionally in the mouth. The altered conditions under these circumstances may account for the difference in morphology².

The colonies of the typical and atypical forms of the Hofmann's bacillus on serum are indistinguishable from those of diphtheria.

Subcultures were made from colonies of Hofmann's bacillus containing some, often a considerable number, of long segmented bacilli from nearly 100 persons. In every case in subculture the typical form of the bacillus alone, or combined with a few long forms, was seen, and all that were tested produced an alkaline reaction in glucose broth.

The different reactions produced in glucose broth by the growth of diphtheria and Hofmann's bacilli have already been compared (p. 278), and the cultural differences on potato-agar fully detailed, the former producing colonies of one of the two types recorded and the latter round, smooth, dome-shaped colonies without distinctive features.

Cobbett (iv. 1901, p. 243), in a former outbreak in this town, isolated and tested for virulence 69 specimens of this bacillus, derived from persons recovering from the disease, contacts and normal people, and all without exception were completely devoid of virulence in doses of 2 c.c. injected subcutaneously and produced an alkaline reaction in glucose broth. All examples which I have previously tested have

¹ Graham-Smith (1903, p. 230).

² This organism differs from the *Bacillus coryzae segmentosus* described by Cautley (1896) and which has been also found by myself in similar circumstances (p. 302).

behaved in a similar manner. In consequence of these observations I have only thought it necessary to test for virulence specimens obtained from persons suffering from the disease in early stages, infected contacts, and cases of suspicious sore throat in which no other diphtheria-like organisms had been found. The negative results of these experiments have already been given (p. 292). More than 150 examples of the pseudo-diphtheria bacillus were, however, also isolated from convalescents, contacts and normal persons, and grown on various media. All without exception produced an alkaline reaction in glucose broth, and behaved in other respects after the characteristic manner of these bacilli.

The investigations of nearly all observers show that both amongst contacts and normal children attending public schools the percentage of infection with the pseudo-diphtheria bacillus is very high, owing to the fact that sweets, slates, pencils, etc., pass from one child to another, and to the habit children have of placing their fingers, and such articles as pencils in their mouths. The absence of such habits amongst older persons may account for the smaller percentage of infection amongst them with this bacillus.

During the outbreak at Colchester (1901) I examined 563 cultures from contacts of all classes not infected with the diphtheria bacillus, and found the bacillus of Hofmann on 316 occasions (55·4 %). The great majority of the persons examined belonged to the poorer classes, and the bacillus was of very frequent occurrence in their throats, whereas in the more well-to-do the percentage of cases in which it occurred was much lower. In schools attended by the children of the poor where many articles were shared in common, and want of strict attention to cleanliness was frequently observed, 64·5 % harboured this bacillus.

In the following table it will be noticed that there was no relationship between the percentage of persons infected with Hofmann's bacillus and that infected with the diphtheria bacillus. Almost all the persons mentioned in the second half of the table were adults of the well-to-do class who had not been in contact with diphtheria. The percentage of infection with the pseudo-diphtheria bacillus amongst them was small (19 %), for the reasons which have been explained, and is only slightly less than that (22·7 %) found in well-to-do persons at Colchester amongst whom a large number were infected with the diphtheria bacillus.

TABLE VI.

The table gives the percentage occurrence of Hofmann's bacillus amongst school children and others at Colchester, and amongst healthy non-contacts, the majority belonging to the well-to-do class, at Cambridge.

Class of persons examined			No. of cultures	Percentage infected with Hofmann's bacillus	No. of healthy persons infected with diphtheria bacilli*
<i>Colchester</i>					
Scholars.	School	I	6	66.6	0
		II	30	66.6	0
		III	50	64.0	8
		IV	49	63.3	8
		V	149	63.0	6
		VI	59	62.7	5
		VII	16	62.0	1
		VIII	15	60.0	5
		IX	37	57.0	5
		X	9	33.3	1
		XI	10	30.0	0
Persons above school age			40	50.0	3
Well-to-do persons			79	22.7	23
<i>Cambridge</i>					
Scholars.	School	XII	29	0	0
		XIII	49	12.2	1
Patients in Addenbrooke's Hospital			98	12.2	1
Undergraduates of Sidney Sussex College			41	21.9	3
Members of the University			48	4.1	0
Workers in the Pathological Laboratory			18	22.2	0
Other persons			198	24.7	0
Total			1044	36.4	70

* Not included in the numbers of examinations.

The next table shows that the percentage infection with Hofmann's bacillus is very variable, according to these observers ranging from 78.9 to 5.7%. It again, however, demonstrates that the proportion of persons infected with Hofmann's bacillus bears no relation to the proportion infected with the diphtheria bacillus. Taking the school children alone it is seen that nearly one-third harbour the pseudo-diphtheria bacillus in their mouths.

TABLE VII.

The results of observations by various investigators on the occurrence of Hofmann's bacillus in healthy persons.

Observer	No. of persons examined	No. of times Hofmann's bacillus found	Percentage infected with Hofmann's bacillus	Percentage infected with the diphtheria bacillus	Remarks
Cobbett (1903)	1495	536	35.8	?	Mostly school children
	19	15	78.9	5.0	Scholars, children of the poorer class, attending three schools
	49	37	75.5	0	
	120	76	63.3	6.6	
Minnesota Board of Health (1901)	30	21	70.0	3.3	Mankato School, p. 533
	50	30	60.0	6.0	Faribault „ p. 526 (feeble minded)
	40	22	55.0	5.0	Owatonna „ p. 531
	57	22	38.6	10.5	Faribault „ p. 529 (public schools)
	193	52	25.9	13.7	Owatonna „ p. 519
	242	31	13.2	28.5	Bethany Home, p. 535
	24	2	8.3	25.0	Albert Lea Schools, p. 522
Chatin & Lesieur (1900)	75	22	29.3	2.66	School
Hewlett & Murray (1901)	385	92	23.9	15	Children admitted to a General Hospital
Berry & Washbourn (1900)	142	33	23.2	12	Schools
Goadby (1900)	586	99	16.9	31.4	„
W. Pakes (1900)	3000	446	14.8	14.3	Sore throats
Park & Beebe (1)	330	27	8.2	0	Healthy persons, see p. 262
Herman Biggs	330	19	5.7	10	„ „ „ p. 261
Total	7233	1624	22.4		
	(3265)	1059	32.1		School children

From the following table (VIII) it will be seen that the infection with Hofmann's bacillus amongst the scholars of the public schools in this outbreak was very high, ranging from 72 to 35%. It is particularly interesting to note that the school which showed the greatest infection with the diphtheria bacillus showed the lowest infection with Hofmann's bacillus, and that schools in which no virulent diphtheria bacilli were found showed a very high degree of infection with Hofmann's bacillus. *This table again demonstrates that there is no relation between the numbers infected with virulent diphtheria bacilli and those infected with pseudo-diphtheria bacilli.* This point is made especially clear in the case of the Catherine Street School where the percentage of infection with Hofmann's bacillus in all five classes is practically identical, whereas in only three were diphtheria bacilli, virulent or non-virulent, found.

TABLE VIII.

Showing the proportion of persons infected with pseudo-diphtheria bacilli and with virulent and non-virulent diphtheria bacilli in the various schools and institutions examined during this outbreak.

Schools	No. of persons examined	No. in whom Hofmann's bacillus found	Percentage infected with Hofmann's bacillus	Percentage infected with virulent diphtheria bacilli*	Percentage infected with non-virulent diphtheria bacilli*
Sturton Street School	120	72	60.0	4.1	.8
Ross " "	46	28	60.0	2.1	0
Abbey School	32	19	59.3	0	3.0
New Street School	42	22	52.3	0	2.3
Catherine Street School					
Class i	47	25 (53.1)	51.1	0	0
" ii	102	50 (49.0)		1.0	0
" iii	54	28 (51.8)		14.0	1.5
" iv	59	27 (45.7)		3.1	3.1
" v	41	26 (63.4)		0	0
Park Street School	20	10	50	0	0
St Matthew's School					
(March)	288	105 (36.4)	37.4	5.5	1.2
(Autumn)	49	24 (48.9)		14.0	0
Girls	122	43 (35.2)		6.1	.7
	1022	479	46.8	4.8	1.07
Dress-making establish- ment	29	7	24.1	3.0	0
Sanatorium	49	10	20.4	15.5	0
Post Office	52	8	15.3	1.8	1.8
Hospital	40	6	15.0	17.5	2.2
Printing Office	19	2	10.5	0	0
	189	33	17.4	8.5	.9
Notified persons with diphtheria bacilli	52†	25	48.0	50.0	11.3
Infected contacts	52†	27	51.9		32.7
Suspicious cases	79	13	15.4		—
Total	1409	577	40.9	6.4	1.8

* These persons have not been included in the totals given under each head, but are given separately near the end of the table.

† Only those persons have been included here in whom two or more examinations have been made. In the totals, however, all persons examined have been included.

Further it is again shown that amongst adults a much smaller proportion harbour the pseudo-diphtheria bacillus in their throats, only 17.4% showing them as against 46.8% of the children, although 8.5% of the former were infected with the virulent diphtheria bacillus as against 4.8% of the latter.

A point of still greater interest in view of the statements which have been made is the proportion of persons infected with Hofmann's bacillus amongst the notified cases and contacts harbouring the diphtheria bacillus. In these two classes 48 % and 51.90 % respectively showed at some period pseudo-diphtheria bacilli in cultures from their throats.

Most of these persons were children attending the public schools, and it is seen that the total proportion infected (50 %) with the pseudo-diphtheria bacillus almost coincides with the proportion infected with this organism amongst the healthy scholars attending the same schools (46.7 %). These figures are all the more striking when it is remembered that each of these persons was examined on many occasions and the opportunities for finding the pseudo-diphtheria bacilli if they existed in their throats were very great. The results of each examination in these two classes are given in the tables at the end, and an inspection of these makes it clear that in some cases the pseudo-diphtheria bacilli were found throughout, whereas in other cases they were never found.

From these observations I am of the opinion that this bacillus is not more frequently found amongst the latter class of persons than amongst normal persons, and that it does not replace the diphtheria bacillus when that organism is disappearing, but that it is either present or absent in the cases under examination throughout the whole period¹.

"In view of the wide distribution of Hofmann's bacillus amongst healthy persons in Cambridge and elsewhere the conclusion arrived at by Richmond and Salter that the pseudo-diphtheria bacillus is a variety of the true causal agent of diphtheria is, if well founded, of great importance. But until the position of the bacillus of Hofmann has been established and it has been found capable of being converted into the virulent diphtheria bacillus, not only by laboratory procedures but further under natural conditions, we must not conclude that the causal agent of diphtheria is widespread" (Cobbett, iv. 01, p. 247).

¹ It must be remembered that at the time when some of these examinations were made the pressure of work was very great, consequently time could only occasionally be spared to look for the pseudo-diphtheria bacillus once the diphtheria bacillus had been found in the culture. Hence the omission to state that the former organism was present when the latter was found cannot be taken to mean that it was not present in the culture under examination. In cases, however, in which several examinations had been made without finding the Hofmann's bacillus special care was taken to thoroughly examine subsequent cultures for its presence.

These observations, I think, confirm the statements I have already made in regard to this organism (1903, p. 250), namely:

(1) That the bacillus of Hofmann (as previously defined) is perfectly innocuous to man.

(2) That it is a common inhabitant of the mouths of the poorer classes, especially children.

(3) That it is relatively uncommon amongst adults, both of the poorer and well-to-do classes, and even amongst the children of the latter class.

(4) That it is probably spread from one child to another by the means that have been indicated as the probable ones by which diphtheria bacilli are transferred from one individual to another (1903, pp. 238—241).

(5) That in the absence of diphtheria bacilli morphologically resembling Hofmann's bacillus, described by Wesbrook in an outbreak at Owatonna, but which have never been met with by Cobbett or myself, no importance whatever should be attached to the presence of Hofmann's bacillus.

*Organisms morphologically resembling diphtheria bacilli
on serum cultures.*

(a) From the nose and throat.

Apart from Hofmann's bacillus which only presents difficulties in diagnosis when giant forms, or the pseudo-diphtheria type alone, are present, various diphtheroid organisms have been described from the throat and nose.

Attention here has only been paid to those which grow on serum, and which bear a close resemblance to the diphtheria bacillus, when cultivated on that medium¹.

Cautley (1896) has described an organism which he has named the *Bacillus coryzae segmentosus*, obtained from the nasal secretion of seven out of eight cases of "Influenza Cold." Gordon (1901) has also isolated this organism, and has supplemented Cautley's account of it.

On serum after 18 hours' growth at 37° C. the colonies are very small. The organisms are of medium length and segmented. Some clubbed forms are present. They retain Gram's stain, and show polar bodies by Neisser's method. On gelatin at 20° C. small round grayish-white colonies are produced, and on agar

¹ The cultural characters in which these organisms markedly differ from diphtheria bacilli are in italics.

at 37° C. transparent colonies, which become grayish-white, and eventually filmy at the edges. *Broth* remains clear and growth chiefly takes place in small flakes forming a *scanty filmy deposit*. On all these media the bacilli are short and oval. In *dextrose broth* a *feeble acid reaction* is produced, and the same is the case in litmus milk, but clotting does not occur. It is non-pathogenic to guinea-pigs.

Cautley also isolated an organism from the mouth, only differing from the above in producing a flocculent deposit in *broth*, which becomes granular on shaking; and another from the nose differing in producing a thick white growth on *agar*, and turbidity and much white deposit in *broth*. This author appears not to have grown his bacilli on potato or glucose broth, nor to have made animal inoculations.

Gordon (1901) also isolated four other species of diphtheria-like bacilli.

No. 6. Grows well on *serum* in 18 hours at 37° C., but the organisms are much smaller than diphtheria bacilli, though they are segmented and clubbed. They retain Gram's stain, and show polar bodies by Neisser's method. On *agar* there is a good raised white growth, but the bacilli have no polar bodies. There is no growth on *gelatin*. *Broth* becomes slightly turbid and *conglomerate flocculi* are formed at the bottom of the tube. In *dextrose broth* no acid is produced. *Litmus milk* becomes alkaline and no clotting occurs. It is non-pathogenic to guinea-pigs.

Nos. 7 and 8. On *serum* produce *cokerent growth*, but give rise to an acid reaction in *glucose broth*. No. 7 shows polar bodies by Neisser's method, but No. 8 does not.

No. 9 was isolated on four occasions from the throats of persons suffering from diphtheria.

On *serum* in 18 hours growth is moist, gray and raised, but not copious. Great variations occur in the shape of the organisms, every gradation between oval and bloated pear-shaped forms being found. They retain Gram's stain, and show polar bodies by Neisser's method. On *agar* round, gray, raised colonies develop which *adhere* to the medium. The colonies on *gelatin* are gray and in 10 days the medium begins to be *liquefied*. *Broth* shows a slight turbidity and a *weedy sediment*. Acid is formed in *dextrose broth*. *Litmus milk* becomes acid, and is *clotted* on the sixth day. It is non-pathogenic to guinea-pigs.

Excellent illustrations accompany the papers of these two authors.

Abbott (1891) discovered in the mouth an organism indistinguishable from the non-pathogenic diphtheria bacillus except in the fact that it formed a *dirty brown layer on potato*.

Davis (1898) isolated from the mouth in certain cases of scarlet fever a short diphtheria-like non-motile bacillus which in doses of 2 c.c. subcutaneously produced a general *septicaemia* and peritonitis in guinea-pigs. On *agar* it produced colonies with *dark centres and uneven edges*, and an acid reaction in *glucose broth*. A. Williams (1898) also seems to have isolated the same species from a patient who had suffered from diphtheria six weeks before.

Ruediger (1903) has recently described an organism, which he calls a virulent pseudo-diphtheria bacillus.

Seven strains of this organism were isolated from seven fatal cases of scarlet fever with gangrenous tonsillitis. Diphtheria antitoxin had no influence on the patients, and in fact in two cases its injection appeared to hasten death. The bacillus is described as resembling the true diphtheria bacillus in morphology, causing "*uniform turbidity in broth*, a soft, moist, and whitish growth on *agar*, a *hardly light brown* growth on *potato*, and turning *litmus milk white* in 5—6 days." Apparently all strains showed polar bodies by Neisser's method, and were agglutinated in dilutions of 1 in 200 by the serum of a rabbit which had received three injections of 24 hour broth cultures of strain No. 5. "Guinea-pigs are *not protected* against this organism *by anti-diphtheria serum*. All 7 strains are pathogenic for guinea-pigs after having been kept on agar for several months, when injected intraperitoneally in doses of 4 to 5 c.c." At the autopsies "the serous cavities contained a moderate quantity of fluid. The liver, spleen, and kidneys were markedly congested. The organisms could be isolated from the peritoneal cavity, heart's blood, and internal organs." A protective serum for guinea-pigs against injections of living cultures was obtained from a rabbit.

The author makes no mention of the reaction of his cultures in sugar media and appears to use the term pseudo-diphtheria bacillus as equivalent to the non-virulent diphtheria bacillus.

*Diphtheria-like organisms isolated from the mouth and nose
during this outbreak.*

Owing to the confusion produced by the method of simply describing micro-organisms without the application of a scientific name, I have decided even at the risk of the possible creation of synonymic titles to give names to the bacilli, whose characters are described in the following pages, choosing ones which indicate some marked peculiarity in morphology or growth, or the source from which the organisms were obtained.

Note. In the following descriptions the cultural characteristics in which the organisms decidedly differ from the diphtheria bacillus are marked by italics.

Some of these organisms have now been cultivated on artificial media for nearly a year. All have been grown on several (6—8) occasions on each of the media on which their characteristics are noted.

Owing to the fact that the precise measurements of bacteria are but of little use in diagnostic work, the terms "long," "short" and "of medium length" are used to describe the bacilli. The term "bacilli of medium length" indicates organisms about the length of the Hofmann's bacillus (*i.e.* about $1.5\ \mu$) and the term "short," organisms considerably shorter. The term "long" is used of organisms about the usual length of the diphtheria bacillus ($3\text{--}6\ \mu$) or longer.

By "large" colonies are meant colonies over .15 cm. in diameter, by "medium-sized" ones about .1 cm., and by "small" colonies considerably under .1 cm. in diameter. The colonies are described as seen under a $5\frac{1}{2}\times$ Steinhilber lens.

Unless otherwise stated the growth in sugar-free and glucose broth was similar.

In testing the reaction of glucose broth after 48 hours' growth at 37°C., the indicator used was neutral litmus.

Throughout these descriptions the term "non-pathogenic" means that 2 c.c. of 48 hour cultures in sugar-free broth injected subcutaneously were without effect on half-grown guinea-pigs during the period they were under observation (14—21 days).

All media with the exception of gelatin were cultivated at 37°C., the latter at 22°C.

All these organisms were tested for their power of reducing nitrates. Unless mentioned no reduction was found after 10—20 days' growth.

Spore formation has not been observed in any of these organisms.

1. *Bacillus coryzae segmentosus* Cautley.

Origin. Obtained from the nasal secretions of three persons suffering from coryza.

On serum grows more slowly than the diphtheria bacillus, though the colonies are very similar in appearance. *Organisms* in 24 hours resemble pseudo-diphtheria bacilli with light median bands, but are longer. A considerable number of long forms resembling uniformly stained diphtheria bacilli (Plate XIV, Fig. 6) are also found. They are non-motile, retain Gram's stain, and show small terminal polar bodies in most specimens. In *subcultures* many long segmented forms are seen, with two to four well stained segments, as well as shorter Hofmann-like forms. On agar in 48 hours *round, smooth, white, raised, dome-shaped colonies* are formed. The organisms are long, well segmented, curved and clubbed, and but few short forms occur. Polar bodies are only found in a few. They resemble closely the diphtheria bacillus shown in Plate XIV, Fig. 5. In *agar stab* cultures in 24 hours a small white round smooth surface growth is formed, which is very extensive after 48 hours. There is well marked growth in the depth. On *alkaline potato-agar* in 24 hours very small *round, smooth, gray, dome-shaped colonies* occur, which in 48 hours are *large, smooth, white and well raised above the surface* (like Plate XVII, Fig. 5). On *acid potato-agar* a similar growth occurs. On *gelatin* at 22°C. small round white colonies are produced. On *potato* an almost invisible thin whitish growth is formed. *Broth* remains clear, and a *stringy white deposit* is found at the bottom of the tube. *Glucose broth* in 48 hours is *very faintly* acid. Non-pathogenic.

The examples of this organism which I have isolated and tested agree in their characters with the descriptions given by Cautley and Gordon for the *B. coryzae segmentosus*. They differ from diphtheria bacilli in their rate of growth on serum, the characters of their growth on agar, potato-agar and broth, and in the degree of acid formation in glucose broth. In morphology the majority of specimens resemble the Hofmann's bacillus rather than the diphtheria bacillus, but the resemblance to the latter is striking in some of the larger forms.

2. *Bacillus diphtheroides citreus*. (Plate XV, Fig. 9.)

Origin. Obtained from the throats of five healthy children, two attending infected, and three non-infected, schools.

On *serum* in 24 hours the colonies closely resemble those of the diphtheria bacillus, but are rather larger and more opaque. After three days' growth they become slightly yellowish. The *organisms* are fairly long and stain darkly, in shape resembling Hofmann's bacilli, but with little trace of a median band. The majority are slightly curved, and show small terminal polar bodies, though in some of the longer forms they are very large and distinct. Non-motile, and retain Gram's stain. In 48 hour subcultures a number of long forms with three or four well marked segments and distinct polar bodies occur. On *agar* in 24 hours *large, white, round, smooth, moist, dome-shaped colonies* are formed. The organisms are the same in appearance as on serum, but the polar bodies are better marked. In *agar stab* cultures a flat, thick, moist, white surface growth with indented edges is formed. The indentations are very evident after 48 hours' growth. Along the needle track the growth is well marked. On *alkaline potato-agar large, round, white, smooth, dome-shaped colonies* are formed (like Plate XVII, Fig. 5). On *acid potato-agar* the growth is similar. *No growth* was obtained on *gelatin*. On *potato* in 24 hours a *very extensive pale yellow moist growth* occurs. The organisms are mostly short, but a number of markedly clubbed and segmented (up to 6 or 8 segments) forms are found. All show large and distinct polar bodies. *Broth* after 48 hours is clear with *white rather stringy deposit*. In *glucose broth* the deposit is copious, and white in *large flocculent masses* which tend to stick to the sides. The reaction is very acid. Non-pathogenic.

This organism differs slightly from the diphtheria bacillus in morphology though somewhat resembling Type 5 (Plate XIV, Fig. 6). It also differs in its growth on agar, potato-agar, broth and potato. It is from its marked growth on the latter that the name has been given. It resembles the organism next described very closely except in its growth on gelatin, potato, broth and glucose broth. It may be the same organism as that described by Abbott (1891).

3. *Bacillus diphtheroides brevis*. (Plate XV, Fig. 10.)

Origin. Obtained from a large abscess cavity opening into the mouth.

The colonies on *serum* in 24 hours are small, smooth, and white, but not so well raised as those of the diphtheria bacillus. Subsequently they develop somewhat filmy edges. The *organisms* in shape resemble Hofmann's bacilli, but are slightly curved and clubbed and show segmentation. The segments stain darkly, but the intervening bands except the middle one are not very definitely marked. They are non-motile, retain Gram's stain, and show polar bodies by Neisser's method. In subsequent subcultures segmentation is a marked feature. On *agar* stroke cultures

an *abundant, white, soft, slimy* growth is produced in 24 hours. The organisms are long, well curved, clubbed and segmented, and show good polar bodies resembling the diphtheria bacillus shown in Plate XIV, Fig. 3. *Agar stab* cultures show abundant white slimy surface growth which frequently becomes after further growth coarsely granular. A confluent, abundant growth occurs along the needle track with numerous projecting colonies. On *alkaline potato-agar* in 24 hours *large, round, smooth, gray, dome-shaped* colonies are formed, which in 48 hours are white and considerably larger (like Plate XVII, Fig. 5). The organisms vary from short oval to long well segmented forms. On *acid potato-agar* the colonies are similar. On *gelatin* in three days medium-sized, round, smooth, dry-looking colonies with raised centres develop. On *gelatin stab* cultures a large, white, dry, granular surface growth is formed, and small round colonies develop in the needle track. On *potato* in 24 hours an *extensive growth, thick, soft, and cream-coloured* is formed which gradually becomes granular and slightly *yellowish*. The organisms vary in appearance, some are short, but many long and well segmented with good polar bodies. In 48 hours *broth* becomes slightly cloudy and there is a fine whitish granular deposit. The reaction of *glucose broth* is extremely acid. *Milk* becomes *partially coagulated* in a few days and *indol* is *produced*. It is non-pathogenic.

This organism differs from the diphtheria bacillus in its growth on agar, potato-agar and potato, and somewhat in its morphology.

4. *Bacillus maculatus*. (Plate XV, Fig. 11.)

Origin. One colony found in a culture from the throat of a possible contact.

On *serum* in 24 hours the colonies are opaque white, but otherwise resemble those of the diphtheria bacillus. The organisms are longer and broader than diphtheria bacilli, but some short forms occur. The sides in some have slight bulgings at intervals. Numerous darkly stained segments cross the bacillus transversely in most of the organisms, but in a few there are oval segments. In some bacilli, especially in the later subcultures, long unstained intervals are seen. The organisms are non-motile, and retain Gram's stain very deeply. Each bacillus shows *numerous polar bodies* by Neisser's method; some of these are large and round, others *elongated transversely* across the bacillus, whilst others are *very minute*. These minute polar bodies are often very densely aggregated. The name indicates the remarkable spotted appearance seen when the organisms are stained by Neisser's method.

In the first culture the organisms lay in tangled masses of 10—50 individuals. On *agar* in 24 hours minute, round, transparent colonies are formed which subsequently grow very slowly. The organisms are about one-third the length of those found on serum cultures and of various shapes, from oval to bloated pear-shaped bodies. The polar bodies are few. On the surface of *agar stab* cultures an almost transparent film is formed, but in the depth medium-sized yellowish colonies grow. On *alkaline potato-agar* rounded, small, rather flat, slightly granular colonies with irregular edges are produced (Plate XVII, Fig. 6). The growth on *acid potato-agar* is similar. On *gelatin* after 10 days' growth the colonies are so *minute* as to be scarcely visible with a lens. Exceedingly

minute colonies also form in the depth of *gelatin stab* cultures. On *potato* there is no visible growth. *Broth* remains clear, but a *few very large, discrete, yellow granules* (0.5 cm. in diameter) are seen after 48 hours. The reaction of *glucose broth* becomes *very faintly acid*. *Milk* remains unchanged and no *indol* is formed. The organisms are non-pathogenic when injected either subcutaneously or intraperitoneally.

This organism differs from the diphtheria bacillus slightly in its morphology, its growth on potato-agar and gelatin, and in the very large size of the granules formed in broth, and the degree of acid formation in glucose broth.

5. *Bacillus diphtheroides liquefaciens*. (Plate XV, Fig. 12.)

Origin. Found in considerable numbers in the mouth of a patient suspected to be suffering from diphtheria.

On *serum* minute rounded colonies are formed in 24 hours, but in 48 hours they are medium-sized, round, slightly yellowish, dome-shaped and opaque. After 10 days' growth the colonies have sunk into slight pits, and the medium become *partially liquefied* after being kept 20—30 days at room-temperature. The *organisms* are very long, and markedly curved, and lie in groups more or less parallel to one another. There is very little clubbing, and but slight signs of segmentation, but all show well marked terminal and other polar bodies. Some specimens remain as unstained shadows. They are *motile*, but the movements are slow, and they retain Gram's stain. These organisms bear a fairly close resemblance to the diphtheria bacilli shown in Plate XIV, Fig. 5. On *agar stroke* cultures in 24 hours a *thick, moist, smooth, slightly yellow abundant growth* is formed. The appearance of the organisms is the same as on serum. On the surface of *agar stab* cultures an extensive moist smooth growth occurs, which occasionally in old cultures shows concentric markings. In the depth the colonies run together to form a continuous growth, the discrete colonies at the edges are rounded, but have blunt projections. On *alkaline potato-agar* in 48 hours round *smooth, moist, dome-shaped, slightly yellowish colonies* (like Plate XVII, Fig. 5) are formed, and the same is the case on *acid potato-agar*. On *gelatin* very minute, almost transparent colonies are formed. The medium becomes *liquefied* round them in about 10 days. In a few more days liquefaction is complete with a whitish-yellow mass lying in clear fluid. On *gelatin stabs* in 3 days the small yellowish surface growth is lying in a small cup-shaped area of liquefaction. In 11 days there is a *deep funnel-shaped hollow* with yellowish growth at the bottom and very minute colonies along the lower part of the needle track. On *potato* in 3 days a *thin extensive white growth* is formed, which in 6 days is *very abundant and yellow*. The organisms are of medium length, markedly curved, thin and stain uniformly. Many are clubbed and the polar bodies are very minute. *Broth* in 48 hours is slightly cloudy, with a large deposit of finely granular matter. In 48 hours the reaction of *glucose broth* is *neutral or faintly alkaline*. In 6—8 days *litmus milk* is *decolourized* and *firmly clotted*. No gas is produced, but much *indol* is formed and *nitrites reduced*. It is non-pathogenic.

This organism closely resembles some forms of the diphtheria bacillus, but differs in its growth on agar, potato-agar and potato, in the liquefaction of serum and gelatin, its reaction in glucose broth and its action on milk.

(b) *From the conjunctiva.*

Virulent diphtheria bacilli have been isolated from cases of diphtheria of the conjunctiva by Gordon (1) (1901), Jessop (3) (1895 and 1902), Eyre (1) (1897) and others.

An organism resembling the diphtheria bacillus very closely in many respects, known as the Xerosis bacillus, has been obtained from the conjunctival sac, in health and disease, by many observers.

Kuschbert and Neisser (1884) found the xerosis bacillus in large numbers in xerosis conjunctivae, Eyre (1897) in 12 persons with conjunctivitis, and many others have also found it in diseased conditions. Opinions however differ as to whether it is to be found in the healthy eye. Eyre (1897) examined 25 normal eyes, but could not find it, Stephenson (1898) out of 6209 normal children found it in 1·87 %, whereas Uthoft (1893) states that the xerosis bacillus is frequent in normal eyes and Lawson (1899) found it in 74·2 % of 200 persons examined (in 90 in pure culture). The latter observer considers it to be the most common and most universal inhabitant of the conjunctival sac.

Opinions also differ as to whether the xerosis bacillus is a non-virulent diphtheria bacillus, or a distinct species. Fraenkel (1896) inclined to the former view, but most of the other observers just quoted to the latter.

Eyre (1897) states that in the original cultures colonies only appear on *serum* after 48 hours, but in subsequent cultures grow well. The colonies are described as opaque, slightly heaped up, scaly, and adhering to the medium; and the organisms as resembling diphtheria bacilli in shape and segmentation. They are said not to differ in their growth on agar and gelatin from the diphtheria bacilli. *Broth* after 60 hours' growth remains *alkaline*. The organisms are non-pathogenic, and animals cannot be protected against the diphtheria bacillus by the injection of cultures of this organism.

Stephenson (1898, p. 63) was unable to produce xerosis in healthy human eyes by the inoculation of pure cultures of the xerosis bacillus. This author describes the organisms as retaining Gram's stain very tenaciously (p. 58).

Gordon (1901, p. 425) isolated an organism (No. 3) from a case of conjunctivitis which on *serum* in 48 hours formed a copious growth of *lemon-yellow* colonies. It resembled the diphtheria bacillus in morphology, retained Gram's stain, and showed

polar bodies by Neisser's method. On *agar* there was a copious growth with a *dry surface, and yellow pigmentation*. Growth on *gelatin* was like that formed by the diphtheria bacillus but *yellow*. *Broth* remained clear, and conglomerate yellow crumbs were found at the bottom. In *dextrose broth* an acid reaction was produced. It was non-pathogenic.

In the course of the observations I have examined by culture the conjunctivae of 10 healthy persons, and found the xerosis bacillus in 9. The culture in which it was not found was overgrown with a film-forming organism. Its cultural and morphological characteristics are as follows.

6. *Bacillus xerosis*. (Plate XVI, Fig. 13.)

Colonies only appeared on *serum* in the original cultures after 48 hours' growth and were then very minute. In subsequent cultures, however, the growth is more rapid. The colonies except in size resemble those of the diphtheria bacillus, but tend to *adhere* to the medium, and become irregular at their edges after 72 hours' growth. The *organisms* are of medium length, to long, and are curved, slightly clubbed and segmented. Forms with two to four segments are the commonest. In the original cultures polar bodies are rare, but in subcultures a few specimens show large polar bodies. The bacilli are frequently arranged in small groups. They are non-motile, and retain Gram's stain. On *agar* minute, raised, round, smooth, almost transparent colonies make their appearance in 48 hours. After days of growth very little increase in size takes place. The organisms are broader than on serum, well segmented, but show no polar bodies. In *agar stab* the surface growth is small and almost transparent, and very minute colonies are seen along the line of puncture. On *alkaline potato-agar* small, round, smooth, dome-shaped, gray colonies, resembling those formed by Hofmann's bacillus, appear in 48 hours (like Plate XVII, Fig. 5). The majority of the organisms are short and like Hofmann's bacillus but many irregular forms are seen. On *acid potato-agar* the colonies are similar, but smaller. On *gelatin* only a few of the specimens showed any growth, the colonies being very minute, round, and transparent. In *gelatin stab* cultures only one specimen showed any growth along the needle track, and then only four very minute colonies appeared. On *potato* no visible growth occurred. *Broth* after 48 hours' growth remains clear and a few large granules are found at the bottom. In *glucose broth* the reaction of the cultures after 48 hours' growth varied slightly, being either *very faintly* acid, or neutral. *Milk* remains unchanged. It is non-pathogenic.

This organism resembles the shorter, segmented forms of the diphtheria bacillus very closely, but differs from it in its growth on serum, and potato-agar, in its very poor growth on gelatin, and in its power of producing acid in glucose broth.

As the xerosis bacillus appears to be a common inhabitant of the normal human conjunctiva, cultures were made from the eyes of a few

animals to ascertain whether similar organisms are to be found there. The eyes of 3 dogs, 3 rabbits, and 17 guinea-pigs were examined and organisms closely resembling the xerosis bacillus were found in the eyes of the dogs and guinea-pigs, but not in the eyes of the rabbits.

7. *Bacillus xerosis canis*. (Plate XVI, Fig. 14.)

Origin from the conjunctival sacs of the three dogs examined.

On *serum* the colonies only make their appearance after 2—3 days' growth, though in later subcultures the growth is a little more rapid. After 4—5 days' growth the colonies are of large size. Except for their size the colonies are indistinguishable in appearance from those of the diphtheria bacillus, but *tend to adhere* to the medium. The *organisms* are long, curved, and stain well, showing well differentiated, short, dark segments, separated by narrow light bands crossing the bacillus transversely. Clubbing in some specimens is well marked. These organisms resemble closely the pseudo-diphtheria type of Hofmann's bacillus (Plate XV, Fig. 7). By Neisser's method *variously shaped polar bodies* are seen *in large numbers* in each bacillus. Some are large and round, others elongated transversely across the bacillus, and many are exceedingly small. The organisms are non-motile, and stain well by Gram's method. On *agar* after 2—3 days' growth small grayish colonies with irregular edges, and darker centres appear. On *agar stab* cultures the surface growth is small and almost transparent, but in the depth a few medium-sized round colonies develop. On *alkaline potato-agar* after 2—3 days' growth medium-sized, *whitish, opaque, dome-shaped colonies, with a granular surface and very irregular margins* are seen. The organisms are oval and broad, but show well-marked segments. On *acid potato-agar* the growth is similar. On *gelatin* no growth was obtained. No visible growth occurs in *potato*. *Broth* after 48 hours remains clear but small granules are found at the bottom of the tube, which when shaken up *float in lines* as if held in position by invisible threads. The growth in *glucose broth* is similar, and its reaction after 48 hours is *neutral*. It is non-pathogenic.

This organism differs only slightly from the diphtheria bacillus in morphology but differs from it in its rate of growth on serum and its growth on gelatin, potato-agar and broth, and its reaction in glucose broth. It resembles closely in many respects the xerosis bacillus from the human eye.

14 (82%) out of 17 guinea-pigs' eyes examined showed similar organisms. They resemble the organism just described in morphology, staining characteristics, and in their growth on all media except serum. On serum their colonies are the same in most cultures, but in some cases larger colonies develop which have a raised centre and raised rim resembling the second type of colony formed by the diphtheria bacillus on potato-agar (Plate XVII, Figs. 3 and 4).

These observations indicate that organisms closely resembling the diphtheria bacillus in morphology, and in many respects in cultural

peculiarities, but totally unconnected with them, are common inhabitants of the human conjunctival sac, and that of some animals.

(c) *From the ear.*

Virulent diphtheria bacilli have been isolated from the ear by some observers in a few instances, amongst others by Stevens and Parfitt (1897), and Gordon (1901, p. 424), and others have found organisms morphologically resembling the diphtheria bacillus. Pearce (1898) found such organisms in the middle ear in 25 out of 32 fatal cases of diphtheria. This observer remarks that "it is of interest that many of the cases showed no clinical evidences of ear trouble." Councilman (1893) found at the autopsy organisms which he considered to be diphtheria bacilli in acute inflammatory conditions of the middle ear in one case of diphtheria and two of measles, though the latter had not had diphtheria of the throat. Wright (2) also twice found these organisms under similar circumstances.

Egerton Williams (1901) found organisms which he regards as attenuated diphtheria bacilli in the ears of four children at a fever hospital. In one culture the rods were thick and frequently curved, and in the others short and of various thicknesses. They all formed acid in glucose broth, and were non-pathogenic to guinea-pigs. In conclusion (p. 1803) he says "that when organisms are found at all resembling the diphtheria bacillus they must in the present state of our knowledge be regarded as a modified variety of that organism, bearing in mind that their staining properties are often the only means of diagnosis available, the clinical symptoms being in this class of case often absent, and that these discharges (otorrhoea and rhinorrhoea) unassociated with sore throat, or symptoms, and therefore easily overlooked, may be the cause of unaccountable outbreaks, and the persistence of the disease amongst school children."

Duncan Forbes (1903) amongst 40 cultures from the ears of patients with scarlet fever found in 32 bacilli morphologically indistinguishable from diphtheria bacilli. Most of the organisms were of medium length; in a few cases however long bacilli were found; in some they were quite short, but had the characteristic appearance and staining properties of diphtheria bacilli. This observer does not state that he has made any cultural or virulence tests, but nevertheless regards all these organisms as diphtheria bacilli. Finally he regards it as possible that "if diph-

theria bacilli are present in the air of wards they may readily be carried by air currents."

Welch (1894), however, showed that in many examinations of hospitals diphtheria bacilli were not discovered except in situations which had been infected by direct contact with the patient, or his discharges, and that the bacilli were not present in the air. Hill (1902) recently made some investigations on this subject and confirms Welch's conclusions.

Gordon (1901, p. 424) has also isolated a diphtheria-like organism from the ear of a scarlet fever patient.

It resembles the diphtheria bacillus in morphology on *serum*, retains Gram's stain, and shows polar bodies by Neisser's method. On *agar* in 24 hours *opaque*, *white*, *homogeneous*, *glistening*, and *raised* growth occurs. On *gelatin* the growth is more uniformly consistent throughout, and *more coherent* than that of the diphtheria bacillus. It is non-pathogenic.

It has already been mentioned that in this outbreak a virulent diphtheria bacillus was obtained from the ear discharge of a scarlet fever patient (53, p. 268). This circumstance, together with the fact that diphtheroid organisms indistinguishable from the diphtheria bacillus in morphology were isolated at the same time from other cases, and the improbability of the conclusions at which Williams and Forbes have arrived as to the frequency of the presence of the diphtheria bacillus in the ears of such patients, led me to make some observations on the bacteriology of the ear.

The discharges from the ears of 10 scarlet fever patients were examined by culture, some on several occasions. One harboured virulent diphtheria bacilli, but three had organisms, described below as the *Bacillus auris*, resembling diphtheria bacilli, and three organisms to be described as the *Bacillus ceruminis*, resembling diphtheria bacilli but common in normal ears. Consequently 70 % had organisms morphologically resembling diphtheria bacilli, but differing in other respects.

Twenty normal ears of persons working in the laboratory were also examined by means of swabs, and 13 (65 %) had organisms more or less closely resembling diphtheria bacilli (*B. ceruminis*). Of all the ears examined, therefore, 66·6 % contained diphtheroid organisms.

8. *Bacillus auris*. (Plate XVI, Fig. 15.)

Origin. From the ear discharges of three scarlet fever patients.

On *serum* the colonies closely resemble those of the diphtheria bacillus, but grow more slowly. After 48 hours' growth the colonies are medium-sized. After 30 hours' growth the organisms are of various sizes, the majority of over medium length with darkly staining ends. They stain well, showing well marked segments with intervening light bands. In the longer forms several segments occur. Nearly all, even the shorter forms, are well curved, and have a tendency to be clubbed. A few pear-shaped forms were met with. They are non-motile, retain the stain by Gram's method, and show several well marked polar bodies by Neisser's stain. In subcultures the resemblance to diphtheria bacilli is still more marked. Nearly all the specimens are long, well segmented, and show several good polar bodies. The general arrangement in the field is similar to that of the diphtheria bacillus. On *agar* slopes in 24 hours the colonies are small, round, gray and dome-shaped. The organisms are short, curved and clubbed with well marked polar bodies. *Agar stab* cultures in 24 hours show a smooth, moist, white surface growth, and a good growth of discrete round colonies along the line of puncture. On *alkaline potato-agar* in 24 hours *smooth, gray, round, dome-shaped colonies* appear, which later become large and white (like Plate XVII, Fig. 5). The organisms are long, clubbed, and segmented. On *acid potato-agar* the colonies are smaller, but similar. On *gelatin* small, almost transparent, round colonies are formed in 48 hours, which in 3 to 4 days become *large and white, with a smooth surface slightly elevated in the centre*. The growth is *sticky and tenacious*. The organisms are the same as on serum. On *gelatin stabs* in 3 days there is an irregular, granular, white surface growth faintly marked by concentric rings, and good growth in the depth. On *potato* in 24 hours there is a *slight brownish-yellow growth, which in 48 hours becomes extensive, soft, yellow and glistening*. The organisms are short and oval, and stain well with large terminal polar bodies. *Broth* in 48 hours is slightly cloudy with a *white stringy deposit*. In *glucose broth* a copious, finely granular deposit occurs, and the reaction is very markedly acid. *Milk* remains unchanged. *Indol* is formed. The organism is non-pathogenic.

These organisms closely resemble the diphtheria bacillus in morphology and staining characteristics, but differ, more especially, in their growth on potato-agar, gelatin, potato, and broth.

9. *Bacillus ceruminis*. (Plate XVI, Fig. 16.)

Origin. 13 specimens were obtained from normal ears, and 3 from the ears of scarlet fever patients. 11 of these were fully investigated. The organisms appeared to be more plentiful when ceruminous secretion was present on the swabs. From this circumstance the name has been given.

On *serum* in 24 hours the growth is scarcely visible, but in 48 hours small round colonies, indistinguishable from those of the diphtheria bacillus, are formed. After 72 hours the colonies are medium-sized to large. Two forms slightly different in

morphology were noticed. (a) In 30 hours long thin and curved, and uniformly stained, with small terminal polar bodies. In 72 hours the bacilli are longer, more curved, well segmented, and with well marked polar bodies. (b) Medium length, slightly curved, uniformly stained, but markedly clubbed, with large polar bodies in a few specimens. After 72 hours the appearances of the two forms are similar. These organisms are non-motile, and retain the stain by Gram's method.

On *agar* after 24 hours the colonies are small, round, gray and dome-shaped, but later become large and white. The organisms are of medium length, curved, often clubbed, fairly well segmented, and show good polar bodies. On *agar slabs* a small, white moist surface growth is present in 24 hours, which after 48 hours' growth is often lightly marked with concentric rings. A confluent growth takes place in the line of puncture.

On *alkaline potato-agar* in 24 hours *medium-sized, opaque white, smooth, dome-shaped colonies appear*. On *acid potato-agar* the growth is similar, but more copious. No growth was obtained on *gelatin*. On *potato* in 24 hours *a whitish to yellowish white growth occurs, later becoming abundant and yellow*. After about 10 days the growth has a dry granular appearance. The organisms are mostly short, slightly curved, and clubbed. *Broth* remains clear and a small *stringy, white deposit* is formed. In *glucose broth* the deposit is granular and the reaction *neutral or alkaline*. *Milk* remains unchanged and no indol is formed. They are non-pathogenic.

In morphology and staining characters these organisms closely resemble the diphtheria bacilli shown in Plate XIV, Fig. 6, but differ in the rate of their growth on serum, their characters on agar, potato-agar, potato, and broth, and in producing no acid in glucose broth and no growth on gelatin.

The results of these observations show that a species of non-pathogenic organism, almost indistinguishable from the diphtheria bacillus in morphology, is present in the majority of normal ears, and that another species also resembling the diphtheria bacillus is frequently present in the ear discharges of scarlet fever patients. Under these circumstances it is essential to test thoroughly any organisms isolated from such situations before giving a diagnosis. The hasty generalizations made by two recent observers, Williams and Forbes, as to the frequency of the diphtheria bacillus in the ears of scarlet fever patients are probably based on the finding of the organisms which have just been described. The description of the organisms by the former, as far as it goes, and their lack of virulence correspond with these bacilli. The latter, however, appears to have entirely depended on morphological appearances. Evidence is, therefore, yet lacking that the diphtheria bacillus occurs in the ears of scarlet fever, or even diphtheria, patients with or without symptoms, except in a few instances.

(d) *Diphtheria-like bacilli from birds.*

"Diphtheria of the lower animals, especially fowls, and pigeons, has been made the subject of numerous investigations; and when we examine the literature we are immediately struck with the difference of opinion regarding the disease. On the one side we have those who believe that the one disease in man and birds is identical; and on the other side those who believe that the one disease has no relation to the other" (Harrison, 1903).

Harrison (1903) gives a short summary of the works of various writers and investigators, grouped under two heads:

(1) Those who have investigated the disease as it occurs in fowls and pigeons, by the usual methods employed in working out infectious diseases.

(2) Those who have made observations without experimental research, and who did not employ bacteriological methods to support, or controvert their views, either for, or against, the identity of the disease as it appears in birds and man.

In the first group all the investigators with the exception of Stevenson (1898) found that the disease in birds was due to organisms entirely different from the diphtheria bacillus. The latter, as the result of experiments on diseased fowls with diphtheria antitoxin, stated that "roup," the popular term for fowl diphtheria, was "caused by a specific germ, which appears to me to be identical with the Klebs-Löffler bacillus, and that roup and canker were the same disease, a disease identical with diphtheria in man."

The opinions of the writers recorded in the second group were divided. Harrison (p. 8) inoculated five healthy fowls with human diphtheria bacilli by scratching the throat, and rubbing in 24 hour cultures. Though these cultures were fully virulent to guinea-pigs the fowls were in no way affected. He was unable to infect guinea-pigs or rabbits with the membranes of bird diphtheria. Finally he reports "we have made cultures from over 200 fowls which died of diphtheria, or were killed in certain stages of the disease, but we have not met with the Klebs-Löffler bacillus in any of them, and consequently we cannot believe in the identity of the human and avian disease."

From his own extensive observations Guérin (1901, 1903) has also arrived at the conclusion that avian and human diphtheria are due to entirely different organisms.

With few exceptions, therefore, all the authors who have made extensive experimental investigations on this subject are of the opinion that human and avian diphtheria are different diseases due to different organisms.

Bacilli morphologically resembling diphtheria bacilli have been found in birds by various investigators. Gordon Sharp (1900) found such organisms, and thought that they were diphtheria bacilli, but of less virulence than those found in man. Gallez (1896) had the same experience, and came to the same conclusions. Turner (1900) isolated from birds with diphtheria a bacillus morphologically resembling the diphtheria bacillus. Guérin (1903) in 78 examinations once found a similar organism, and Malvoz (quoted by Guérin) also found one. Macfadyen and Hewlett (1900) isolated and cultivated bacilli, morphologically resembling diphtheria bacilli, from healthy pigeons and others suffering from pigeon "canker."

They describe these organisms as resembling diphtheria bacilli in size and parallel arrangement. They retain Gram's stain, and show polar bodies by Neisser's method. Growth on serum varies, some examples produce dry and abundant growth like the xerosis bacillus, and others moist colonies like the diphtheria bacillus. These organisms produce indol and acid in broth, and are non-pathogenic to guinea-pigs.

Harrison (1901) also found a diphtheria-like bacillus in the throats of normal pigeons.

I have examined by means of swabs the throats of various birds¹ and found the following diphtheria-like organisms.

10. *Bacillus diphtheroides gallinarum*. (Plate XVI, Fig. 17.)

Origin. From the throat of a fowl. There was a hard tumour on the side of the left mandible, but the bird was otherwise normal.

On *serum* the colonies after 24 hours' growth resemble those of the diphtheria bacillus. Later the margins become crenated. In the first cultures the *organisms* were long, curved, and clubbed, with 3 or 4 well marked polar bodies. Slight swellings were present round the polar bodies. The rest of the protoplasm stained lightly, but slight signs of segmentation were present. They resembled closely the diphtheria bacillus shown on Plate XIV, Fig. 5. In subcultures well marked segments

¹ The birds examined were three fowls (*Gallus domesticus*), two Redpolls (*Acanthes rufescens*), two Pied Wagtails (*Motacella lugubris*), two Partridges (*Perdix cinerea*), two Knots (*Tringa canutus*), one Greenfinch (*Ligurinus chloris*), one Cuckoo (*Cuculus canorus*), one Cockateel (*Calopsittacus novae-hollandiae*), one Crested Mynah, and one Indian Ring-necked Parrakeet. The swabs were kindly procured for me by Dr E. Chichester, of Colchester.

are seen. These organisms are non-motile, and retain the stain deeply by Gram's method. On *agar* small, filmy, transparent, gray colonies are formed. The organisms are very long, thick, curved, clubbed, and well segmented, but no polar bodies are present. On *agar stab cultures* an almost transparent film is formed on the surface, and minute round colonies along the needle track. On *gelatin* after five days' growth minute, round, almost transparent colonies appear. On *gelatin stab cultures* there is very little surface growth, and a very scanty growth of minute colonies along the needle track. On *alkaline potato-agar* in 24—48 hours *very small, smooth, or slightly granular, rounded, almost transparent colonies* are formed. On *acid potato-agar* the growth is similar. No visible growth occurs on *potato*. *Broth* remains clear and there is a slight granular deposit. *Glucose broth* shows a *neutral, or slightly alkaline reaction*. *Indol* is produced. Non-pathogenic to guinea-pigs.

As will be seen from the above description this organism very closely resembles the diphtheria bacillus except in its growth on potato-agar, and in the production of an alkaline reaction in glucose broth.

11. *Bacillus cuculi*. (Plate XVI, Fig. 18.)

Origin. Two cultures were obtained, one from the throat of a cuckoo (*Cuculus canorus*), and the other from that of a parakeet.

On *serum* after 24 hours' growth the colonies closely resemble those of the diphtheria bacillus. The *organisms* varied in appearance, some were of medium length, curved, clubbed, and stained throughout (like the diphtheria bacillus Plate XIV, Fig. 6), others resembled elongated Hofmann's bacilli with a central light band. Shadowy unstained forms were common. In subcultures the resemblance to the uniformly stained diphtheria bacillus is more evident. They are non-motile, retain Gram's stain, and show small terminal polar bodies by Neisser's method. On *agar large, smooth, white, dome-shaped colonies* are formed in 24 hours. The organisms are long, much curved, clubbed, and well segmented, but show no polar bodies. On *agar stab cultures* an extensive white heaped up surface growth occurs and numerous colonies along the line of puncture. On *alkaline potato-agar round, smooth, dome-shaped, opaque, white colonies* appear. The colonies on *acid potato-agar* after 24 hours' growth are similar, but after 4 days' growth a *thin, filmy, broad expansion* appears round the central mass. On *gelatin* no growth was obtained, and on *potato* no visible growth occurred. *Broth* becomes slightly cloudy, and there is a finely granular deposit. After 48 hours the reaction of *glucose broth* is *neutral*. Non-pathogenic.

This organism differs to some extent in its morphology from the diphtheria bacillus, and differs from it in its growth on agar, and potato-agar and in its reaction in glucose broth.

These observations confirm those of some of the investigators

mentioned, who found in birds organisms morphologically resembling diphtheria bacilli, but differing from them in certain respects.

The *Bacillus xerosis* and the *Bacillus xerosis canis* are the only ones of all the organisms described which die out rapidly in culture. The others remain alive from 30—60 days on serum at room temperature.

No mention has been made in the foregoing account of certain organisms resembling to some extent the diphtheria bacillus in morphological and staining characters, but which differ markedly in having large wrinkled coherent colonies on serum. In such cases mistakes in diagnosis can only be made if instead of the examination of separate colonies, the surface of the medium is scraped, or smear preparations made from many colonies.

Summary.

1. Diphtheria bacilli have been found in a considerable proportion of persons who have come into contact with cases of diphtheria, or with other infected persons.

2. Such persons have been shown to be a grave danger to the public health, especially when frequenting schools or institutions, and to constitute the usual channel by which the disease is spread.

3. Very satisfactory results have followed on the isolation of convalescents from the disease and of "infected contacts," where two, or more, consecutive negative examinations have been required before release.

4. Carefully conducted investigations amongst healthy persons, who have not at a recent date been in contact with diphtheria cases or infected contacts, have shown that *virulent* diphtheria bacilli are very seldom (2 examples amongst 1511 persons) present in the mouths of the normal population. This fact renders the discovery and isolation of infected persons a practicable possibility, and offers a fair prospect of discovering and isolating the majority of them during any outbreak.

5. Of the 113 examples of the diphtheria bacillus tested for virulence during this outbreak, 87 were fully virulent, and 25 were completely devoid of virulence. One virulent bacillus for reasons explained did not kill the inoculated animal till the 12th day (p. 282). *No partially attenuated bacilli have been found.*

6. In the majority of persons in whom diphtheria bacilli were found, who had recently been in contact with cases of the disease, the bacilli were virulent.

7. Non-virulent bacilli were discovered in 1—2 out of every hundred persons examined, whether contacts or non-contacts. The proportion of persons infected with this organism is therefore the same amongst contacts and persons who have not recently been in contact with the disease.

8. *The absence of polar bodies is no indication of a want of virulence in diphtheria bacilli, and their presence is no indication of the possession of virulence.*

9. Hofmann's pseudo-diphtheria bacillus is a very common inhabitant of the mouths of poorer class children. It is less common amongst adults, even of the same class. *The proportion of persons infected with this organism bears no relation to the proportion infected with the virulent diphtheria bacillus. Notified persons and infected contacts harboured this organism in the same proportions as the healthy school children with whom they had been associated.*

Examples of the Hofmann's bacillus isolated from the first cultures obtained from diphtheria cases were totally non-virulent to guinea-pigs. There is no evidence that it is in any way pathogenic to man.

The distribution of this bacillus points to the conclusion that it is carried from mouth to mouth in the same ways as the diphtheria bacillus, and therefore its widespread prevalence in schools attended by the poorer children is significant, as showing how widely spread and uncontrollable an outbreak of diphtheria may become, unless measures are early taken to deal with infected contacts.

10. Organisms morphologically resembling diphtheria bacilli are not infrequently found in the throats of healthy persons, and require careful examination by culture before they can be identified.

11. The xerosis bacillus is a common inhabitant of the normal conjunctival sac, and organisms closely resembling it are present in the eyes of some animals.

12. Virulent diphtheria bacilli have undoubtedly been found in ear discharges, but *diphtheria-like organisms appear to be extremely common in the ear discharges of scarlet fever patients, and in the ears of normal persons. Consequently no conclusions as to the frequency of the diphtheria bacillus in the ears of scarlet fever patients can be made without the thorough examination of any organisms which may be discovered both by cultural, and virulence, tests.*

13. Diphtheria-like organisms occur in the throats of healthy birds.

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ADDENDUM.

In the middle of April another employee in the Post Office was found to be harbouring diphtheria bacilli. Like those previously discovered amongst these persons the bacilli were non-virulent.

(a) = Notified case. Diphtheria bacilli found.
 (b) = "Contact" infected with diphtheria bacilli.
 Δ = Virulent diphtheria bacillus.
 Λ = Non-virulent diphtheria bacillus.
 * = Hofmann's pseudo-diphtheria bacillus found.
 SS = Small swelling in subcutaneous tissue of guinea-pig.
 MS = Moderate " " " "
 LS = Large " " " "
 + = Death of guinea-pig.

N = No result. Guinea-pig not affected.
 dark red = Suprarenals very dark in colour.
 red = Suprarenals deep red in colour.
 pink = Suprarenals pink in colour.
 present = Pseudo-diphtheria bacilli found on one or more examinations.
 In I, II, III or IV days, i.e. within 24, 48, 72 or 96 hours.

No.	Initials	Results of consecutive examinations	Days during diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations				Results of Autopsy				Pseudo-diphtheria bacillus	
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days				Oedema	Suprarenals		Fluid in pleura
							I	II	III	IV				
St Matthew's School (Girls)														
1	A.S. (a)	O.O.Δ.Δ.Δ.O	72	acid	170	.2	MS	+			extensive (haemorrhagic extensive	dark red	trace	present
2	W.B. (a)	Δ.O.O.O	8	"	170	.2	SS	+				red	-	-
3	G.G. (a)	Fatal. No examinations												
4	K.M. (b)	Λ.O.O.O	8	acid	180 (200 240	.2	N N SS	N N +	N N N	14th day	-	-	-	present
5	G.C. (b)	{Δ.Δ.Δ.Δ.O.Δ.O.Δ.Δ.Δ.O.O. Δ.O.O.O.O	44	"	210	.1	LS	+			extensive	dark red	.8 c.c.	present
6	W.L. (b)	Δ.O.O.O.O	8	"	235	.1	MS	+			"	"	3.2 c.c.	present
7	E.P. (b)	Δ.O.O.O	8	"	180	.2	MS	LS	+		very extensive	"	-	present
8	C.S. (b)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.O	29	"	280	.1	MS	+			extensive (haemorrhagic	"	trace	-
9	R.S. (b)	Δ.Δ.Δ.O.Δ.O.O.Δ.O.O	83	"										
St Matthew's School (Infants)														
10	H.O. (a)	Δ.O.O.O	21	"	not tested						-	-	-	present
11	F.A. (a)	Δ.O.O.O	17	"	230	.1	MS	+			extensive	dark red	4.6 c.c.	present
12	F.E. (a)	Δ.O.Δ.O.Δ.O.O.O.O.O	63	"	160	.2	SS	+			extensive (haemorrhagic extensive	"	-	present
13	F.H. (a)	Δ. Fatal	-	"	170	.2	MS	LS	+			"	-	-

14	M.F. (a)	$\Delta_0 \cdot O \cdot O$	28	acid	190	-2	MS	+					extensive (extensive haemorrhagic	dark red	-9 c.c.	present
16	N.W. (a)	$\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot O$	24	"	430	-3	SS	+					"	"	-	present
16	D.H. (b)	$\Delta \cdot \Delta \cdot \Delta \cdot O \cdot \Delta \cdot \Delta \cdot O \cdot O \cdot O$	29	"	225	-1	MS	LS	+				"	red	3-4 c.c.	present
17	D.E. (b)	$\Delta \cdot \Delta \cdot O \cdot O \cdot O \cdot O$	14	"	225	-1	SS	+					"	red	trace	present
18	V.L. (b)	$\Delta \cdot O \cdot O \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot \Delta \cdot \Delta \cdot O \cdot O \cdot O$	41	"	270	-1	MS	LS	+				"	dark red	-4 c.c.	present
19	M.P. (b)	$\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot O$	20	"	240	-1	SS	+					extensive	"	1-0 c.c.	present
20	T.E. (b)	$\Delta \cdot \Delta \cdot \Delta \cdot O \cdot O \cdot O$	22	"	200	-1	SS	SS	+				slight	red	-	-
21	H.W. (b)	$\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot \Delta \cdot O \cdot O \cdot \Delta \cdot \Delta$ $\Delta \cdot O \cdot \Delta \cdot O \cdot O$	52	"	200 +200	-2 -0	N SS	N N	N N			14th day	-	-	-	present
22	B.T. (b)	$\Delta \cdot O \cdot O \cdot O$	9	"	235	-2	LS	+					extensive (haemorrhagic extensive	dark red	2-3 c.c.	present
23	S.C. (b)	$\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot \Delta \cdot O \cdot \Delta \cdot \Delta \cdot O$ $O \cdot \Delta \cdot O \cdot \Delta \cdot O \cdot O$	51	"	300	-1	MS	LS	+				"	"	-	present
24	G.T. (a)	$\Delta \cdot O \cdot O \cdot O$	17	"	150	-2	MS	+					extensive (haemorrhagic extensive	red	-	present
25	S.M. (b)	$\Delta \cdot O \cdot O \cdot O$	9	"	150	-1	SS	+					dark red	dark red	-	present
26	F.S. (b)	$\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot \Delta \cdot O$ $\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot \Delta \cdot O \cdot \Delta \cdot \Delta$ $\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta$	122	"	180 +210	-2 -0	N N	N N	N N			14th day	-	-	-	-
27	W.O. (b)	$\Delta \cdot O \cdot O \cdot O$	9	"	200	-2	N	N	N			14th day	-	-	-	present
28	A.M. (b)	Δ	-	"	340	-0	N	N	N			14th day	-	-	-	-
29	L.R. (a)	$O \cdot \Delta \cdot O \cdot O \cdot O$	30	"	170	-2	MS	+					slight	dark red	-	present
30	M.H. (a)	Δ . Fatal	-	"	410	-2	SS	MS	+				extensive (haemorrhagic moderate	"	2-5 c.c.	-
31	J.M. (a)	$\Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot O \cdot O \cdot \Delta \cdot O \cdot \Delta \cdot O$ $O \cdot O \cdot O \cdot O \cdot \Delta \cdot O \cdot O$	58	"	210	-2	SS	+					extensive	"	-	-
32	A.M. (b)	$\Delta \cdot O \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta \cdot \Delta$ $\Delta \cdot O \cdot \Delta \cdot O \cdot \Delta$	47	"	250	-2	SS	+					extensive	"	12-0 c.c.	present
33	W.R. (a)	$\Delta \cdot O \cdot \Delta \cdot O \cdot O$	32	"	225	-2	LS	+					"	"	1-0 c.c.	present

+ Bacilli from 28th examination.

+ Bacilli from 7th examination.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of culture in c.c.	Results, Days				Oedema	Supra-renals	Fluid in pleura	
							I	II	III	IV				
34	C.L. (a)	A. Fatal	-	acid	195	.2	MS	+			extensive	dark red	3.2 c.c.	-
35	F.B. (a)	Δ.O.Δ.Δ.Δ.O.O.O.O	27	"	210	.2	SS MS		+		"	"	-	-
36	A.C. (a)	A. Fatal	-	"	220	.2	MS	+			"	red	6.3 c.c.	-
37	R.G. (a)	Δ.Δ.Δ.O.O.O.O.O	25	"	150	.2	SS	+			"	dark red	2.0 c.c.	-
38	J.G. (a)	Δ.O.O.O.O.O	6	"	190	.2	LS	+			"	"	4.0 c.c.	-
Sturton Street School														
39	A.L. (a)	Δ.O.O.O	14	"	260	.1	SS	MS	+		"	"	1.3 c.c.	present
40	G.S. (b)	{ Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.Δ.Δ.O.O.Δ.Δ.Δ.O.O.O Δ.O.Δ.Δ.Δ.O.O.O }	27	"	260	.3	LS	LS	+		(extensive) haemorrhagic	"	6.3 c.c.	present
41	J.T. (a)	{ Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.O.Δ.Δ.Δ.O.O.O }	58	"	210	.2	MS	+			extensive	"	5.5 c.c.	present
42	B.T. (b)	{ Δ.Δ.Δ.O.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O. O.O.O }	62	"	{ 190 + 310 }	.2 2.0	N N	N N	N N	14th day N N N	- -	- -	- -	present
43	M.E. (a)	{ Δ.O.Δ.O.O.Δ.O.Δ.O.Δ.O.Δ.O.O. O.O.O Δ.A.O.O.O.O }	32	"	270	.2	LS	LS	+		(extensive) haemorrhagic	dark red	4.5 c.c.	-
44	S.E. (a)	Δ.A.O.O.O.O	22	"	200	.2	MS	+			extensive	"	5 c.c.	present
Hospital														
45	F.H. (a)	Δ.A.O.O	18	"	200	.2	SS	+			moderate	"	-	-
46	C.B. (a)	Δ.O.O.O.O	15	"	200	.2	MS	+			extensive	"	-	-
47	M.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.O	56	"	220	.2	SS	LS	+		"	"	3.5 c.c.	-
48	R.C. (a)	Δ.O.Δ.Δ.Δ.O.O.Δ.O.O	41	"	275	.2	MS	+			(extensive) haemorrhagic	red	4.8 c.c.	present

	A.R.	(a)	A.A.A.A.A.A.A.O.O.O	51	acid	250	-2	N	N	14th day N	-	extensive	dark red	-	4·5 c.c.	-	present
49	H.R.	(a)	{A.A.A.A.A.A.A.O.O.O. Δ.A.O.O.O}	99	"	250	-2	SS	MS	+							
50	G.R.	(b)	{A.A.A.A.A.A.A.O.O.O. Δ.A.O.O.O}	48	"	250	-2	LS	LS	+		"					
Sanatorium																	
52	A.W.	(a)	Δ.A	-	"	250	-2	MS	+			extensive	"				-
53	T.S.	(b)	Δ.A.A.A.O	44	"	200	-2	SS	MS	+		"	"				-
54	R.F.	(a)	Δ.O.O.O	33	"	210	-2	MS	+			"	"		9·0 c.c.		present
55	D.W.	(b)	Δ. Fatal	-	"	250	-2	MS	LS	+		*	"		5·6 c.c.		-
56	E.H.	(b)	Δ.O.O.O	12	"	320	-2	MS	LS	+		"	"		3·4 c.c.		-
57	B.M.	(b)	Δ.O.O.O	13	"	320	-2	LS	+			(extensive haemorrhagic extensive)	"				-
58	H.H.	(b)	Δ.A.O.O.O.O	12	"	350	-2	LS	LS	+		"	"				-
59	E.H.	(b)	Δ.O.A.O.O.O	10	"	330	-2	LS	LS	+		"	"				-
60	L.B.	(a)	Δ.A.O.O.O	25	"	320	-2	SS	+			slight	red		7·5 c.c.		-
St Matthew's School (Infants—Autumn)																	
61	F.B.	(a)	Δ. Fatal	-	"	300	-3	SS	LS	LS	+	extensive	red		2·5 c.c.		-
62	K.R.	(a)	Δ.O.O.A.O.O.O.O.O	30	"	420	-3	SS	LS	+		"	dark red				-
63	H.H.	(a)	Δ.O.O.A.A.A.A.O.O.O.O	31	"	430	-3	MS	LS	+		"	red		9·7 c.c.		-
64	B.P.	(a)	Δ.A.A.A.O.O.O	27	"	250	-3	MS	+			"	dark red		1·0 c.c.		-
65	L.K.	(b)	Δ.A.A.A.O.O	28	"	450	-3	MS	LS	+		moderate	pink		6·2 c.c.		present
66	H.L.	(b)	Δ.A.O.A.O.O.O *	28	"	450	-3	SS	+			extensive	"		1·2 c.c.		present
67	B.T.	(b)	Δ.A.O.O.A.O.O.O	33	"	400	-3	LS	+			"	dark red				-
68	N.M.	(b)	Δ.A.O.O	57	"	350	-3	SS	+			"	"		2·2 c.c.		present
69	H.M.	(b)	Δ.O.A	?	"	246	-3	MS	+			"	"				-
70	G.P.	(a)	Δ.A.A.A.O.O.O.O.O	18	"	430	-3	MS	LS	+		"	"				-

+ Bacilli from 19th examination.

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See note in text (p. 282).

[†] See note in text (p. 282).

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¹ The size of the publications is given roundly in centimetres.—Ed.

THE INFANTS' MILK DEPOT: ITS HISTORY AND FUNCTION.

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SEVERAL causes have contributed to bring the problems of infantile mortality into greater prominence during recent years. The increased interest in child-study, the growth of humanitarian feeling, and the rapid decline of the English birth-rate during a period of Imperialist expansion have combined to set a higher value upon infant life. In this connection less is heard of the "survival of the fittest" and the "devastating torrent of babies"; the fear of over-population which induced many to regard a high rate of infantile mortality with complacency has faded before the danger of depopulation, and the so-called doctrines of Malthus have given place to the gospel of Fecundity. To those engaged in the work of preventive medicine the continuance of a high rate of infantile mortality in spite of the great improvements in public health administration of the last thirty years is a problem of special interest. The following table shows that although the general death-rate has steadily declined since the early seventies there has been no corresponding decline in infantile mortality, which in fact has increased during 1886-1901. The comparatively low rates in 1902-3 are explained by the exceptional meteorological conditions prevailing during the summer months :—

Years	Death-rate per 1000 population	Deaths under one year per 1000 births
1851—55	22·6	156
1856—60	21·8	151
1861—65	22·5	151
1866—70	22·4	156
1871—75	21·9	153
1876—80	20·8	144
1881—85	19·4	138
1886—90	18·8	145
1891—95	18·7	150
1896—1900	17·6	156
1901	16·9	151
1902	16·3	133
1903	15·4	132

It would appear that there is need for more specialized measures of disease-prevention than those hitherto adopted.

In France, where an unusually low birth-rate has compelled the question of infantile mortality to be regarded as one of national importance, efforts have lately been made to improve the defective methods of infant feeding, which are held to be the chief factor in infantile mortality, by the establishment of organizations having for their object the encouragement of breast-feeding wherever possible, and the supply of sterilized milk for those infants for whom breast-feeding is impracticable. These organizations are of two types, the "Consultation de Nourrissons" and the "Goutte de Lait." The former is the earlier institution, and may be said to date from the year 1890 when Professor Herrgott founded "L'Œuvre de la Maternité" at Nancy¹. The infants born in this institution were required to be brought up by their mothers for medical examination one month after birth, and if the child's progress had been satisfactory the mother received a gift of money². In the years 1890-1900, 2,052 women had passed through this institution and 23,382 francs had been distributed amongst them.

The Nancy maternity charity, however, as established in 1890 was a somewhat embryonic form of the Consultation de Nourrissons; the first fully developed example was founded by Budin at the Charité Hospital, Paris, in 1892³, and two others were afterwards established by him, one at the Maternité Hospital in 1895, the other at the Clinique d'Accouchement Tarnier in 1898. Similar consultations have since been organized by Maygrier, Porak, Boissard, and other accoucheurs in Paris. There are two kinds of Consultations de Nourrissons. Those of the type established by Budin are attached to the maternity hospitals, and are limited to the children born in the hospital. The women are admitted for confinement free of charge, and the children born in the hospital are kept under regular medical supervision for the first two years. Every effort is made to encourage breast-feeding, but where satisfactory evidence can be adduced that this is impracticable, the children are fed on sterilized milk supplied daily at the hospital. Each mother is required to bring her child once a week to the hospital, where it is examined by one of the medical staff. The weight is taken and periodically entered

¹ Variot, *La Goutte de Lait, La Clinique Infantile*, Nov. 1st, 1903.

² Herrgott, *Annales de la Société Obstétricale de France*, 1901.

³ Budin, *Rapport sur les règles à suivre dans l'alimentation du premier âge, Congrès d'hygiène de Bruxelles*, 1903. *Des moyens de combattre la mortalité infantile, Revue Philanthropique*, 1902.

with other particulars in a register. The sterilized milk for the hand-fed children is supplied daily in bottles, each bottle containing sufficient for one meal and no more, and the quantity in each bottle is prescribed by the medical man supervising the child and is regulated by the weight of the child. Hand-feeding is exceptional at the consultations of the accoucheurs¹. At the Clinique Tarnier during the years 1898-1902 no less than 448 of the 527 children who were under observation were fed at the breast.

There are also Consultations de Nourrissons which are not attached to maternity hospitals, but otherwise the work is conducted on much the same lines. The mothers are required to bring their infants regularly for medical examination, and breast-feeding is encouraged by gifts of food and money to those mothers who suckle their children.

The Goutte de Lait, which may be regarded as the precursor and to some extent the prototype of the Infants' Milk Depots in this country, is an offshoot of the Consultation de Nourrissons, and is practically a milk dispensary from which infants are fed under medical supervision upon sterilized milk, with or without modification, supplied at the institution. Breast-feeding is encouraged but usually the great majority of the infants are hand-fed. The Goutte de Lait in fact is mainly intended to succour those infants for whom breast-feeding is practically impossible². The first Goutte de Lait was established by Variot in connection with the Belleville dispensary in 1892, shortly after Budin had organized his Consultation de Nourrissons at the Charité, and according to Variot there are now over 25 of these institutions in Paris.

The first provincial Goutte de Lait, and the first to exist as a separate institution, was established in Fécamp in 1894 by Dr Léon Dufour, who was the first to give the name of Goutte de Lait to these organizations. Since 1894, largely owing to the influence of Dufour and Variot, these institutions have multiplied in France, and there are now over 60 towns provided with one or more Gouttes de Lait. The majority are conducted by philanthropic societies, but in some towns, *e.g.* Nantes and Elbeuf, the Goutte de Lait is a municipal institution. Most of the French

¹ Maygrier, *Les Consultations de Nourrissons*, Paris, 1903.

² Some writers draw a sharp distinction between the Consultation de Nourrissons and the Goutte de Lait. Variot, however, applies the latter term to both organizations; but he lays stress on the difference in type between the consultations at the maternity hospitals where the infants are under medical supervision from birth, and the ordinary Goutte de Lait where most of the children are in a state of ill-health when they are first brought to the institution.

Gouttes de Lait are conducted on the methods laid down by Dufour at Fécamp which are best described in his own words.

Le but de l'œuvre, dès sa fondation, a été celui-ci : lutter contre la mortalité des enfants en bas-âge.

(a) En donnant aux mères de famille tous les conseils et encouragements possibles pour les engager à nourrir elles-mêmes leur enfant au sein.

(b) Toutes les fois que l'allaitement maternel ne peut être fait complètement et réclame le secours de moyens artificiels, l'œuvre fournit du lait de façon que l'enfant reçoive une alimentation mixte.

(c) Quand il est bien avéré que la mère est dans l'impossibilité physique, morale ou sociale, de nourrir son enfant, l'œuvre se charge de préparer elle-même le lait qui est destiné à ce dernier, afin d'éviter les fautes commises, trop souvent, dans cette préparation, et aussi afin d'assurer à l'enfant un lait de bonne qualité dans l'élevage artificiel.

Tous les enfants du premier âge de la ville de Fécamp sont admis à bénéficier de l'œuvre de la Goutte de Lait, sur la demande de leurs parents, de leurs tuteurs ou des personnes ayant la direction et la responsabilité de leur élevage.

Toutefois, son action est principalement dirigée sur la classe pauvre, celle où les difficultés de l'alimentation artificielle sont les plus grandes.

Les enfants sont repartis en trois catégories :

(a) Section gratuite, la première, la base de l'opération.

(b) Section demi-payante.

(c) Section payante.

Les enfants de ces trois sections reçoivent le même lait, préparé de la même manière, et distribué dans un matériel semblable¹.

Chaque enfant a un service de paniers et de biberons double et immatriculé.

Chaque mère de famille reçoit tous les jours, pendant un an, ou plus si besoin est, un panier contenant autant de biberons que l'enfant prend de repas dans un jour de 24 heures (neuf). Ces biberons renferment du lait, en quantité proportionnelle à l'âge de l'enfant.

La remise en est faite contre le dépôt d'un jeton de 0 fr. 10 centimes dans la section des pauvres ; ou d'autres de 0 fr. 30 centimes pour les ouvriers, 0 fr. 50, 0 fr. 75, ou 1 fr. dans la section payante, suivant la situation sociale des parents.

Le lendemain, contre la remise du panier et des biberons vides, un autre service, numéroté également au matricule de l'enfant, est remis contre un jeton de la valeur indiquée ci-dessus.

Une fois chaque semaine, les mères, en venant chercher leur lait, doivent amener leurs enfants à peser, de façon à permettre de contrôler l'évolution de leur accroissement et les soins généraux qui leur sont donnés.

L'œuvre est administrée par :

1. UN COMITÉ DE DIRECTION, composé de : Un Président, médecin, chargé de la surveillance générale, du contrôle de la préparation de lait, de celui du nettoyage des biberons, de la qualité du lait, des pesées, etc.

Une Dame patronnesse, trésorière.

¹ *La Goutte de Lait à Fécamp*, Rouen, 1900.

Trois autres Dames patronnesses.

Ces Dames ont la charge morale de la gestion des fonds octroyés à la Goutte de Lait¹.

The first of these institutions out of France was established in New York City in the spring of 1893 by a well-known philanthropist, the Hon. Nathan Straus.

"This milk depot was located on a pier at the foot of East Third Street, that situation being accessible to a very large tenement-house population. Awnings and seats were put up on the pier so that the babies and their mothers could remain there and inhale the fresh air from the river.

"The building which was erected was, owing to the character of the site, of necessity long and narrow; it was placed several feet from the edge of the pier, so that an outside passage-way connecting the rooms was reserved. The building was divided into four rooms. The first room is used for sterilizing the bottles, stoppers, and nipples, and preparing and pasteurizing the milk. The second room is occupied by large water-baths of iced water for keeping the pasteurized milk until it is delivered. The third room contains ice-boxes for the cans of raw milk. The fourth room, which is nearest the end of the pier, is devoted to the business of selling the milk²."

In the Straus Milk Charity the milk is pasteurized, not sterilized, as in the French depots. It is heated in stoppered bottles to 167° F. (this temperature is reached in about 10 minutes) and remains at that temperature for 20 minutes.

"At first two sorts of milk were furnished: (1) Pure milk pasteurized in eight-ounce bottles; (2) A milk especially prepared for feeding infants, a one-half dilution with water, sugar of milk, and lime-water as follows:

Sugar of milk 12 oz.	Lime-water 8 oz.
Milk 1 gal.	Water 1 gal.

thus producing about what we now speak of as 2.2.7, that is 2% fat, 2% proteids, and 7% sugar. This formula was not considered ideal, but was easily prepared and seemed to answer well. It was dispensed in eight-ounce bottles.

"Later, on the advice of Dr A. Jacobi, there was added a one-half dilution of milk with barley-water which was sweetened with cane-sugar according to the following formula:

Table-salt $\frac{1}{4}$ oz.	White cane-sugar 10 oz.
Milk 1 gal.	Barley-water 1 gal.

This was dispensed in six-ounce bottles.

"The six-ounce bottles of both the prepared milks were sold at one cent each. The eight-ounce bottles of pure milk were sold at one and a half cents each³."

¹ Dufour, *Comment on crée une Goutte de Lait*, Fécamp, 1902.

² Rowland G. Freeman, *The Straus Milk Charity*, New York, 1895.

³ Freeman, *Milk Pasteurization*, New York, 1897.

Mr Straus endeavours to obtain as pure a milk supply as possible, the milk is certified by the New York Milk Commission and the farms and cows from which it is derived are inspected by the Veterinarian of the New York Board of Health. In 1902 the Straus Milk Charity had 14 depots at work in New York, and 1,200,000 bottles of milk were supplied¹.

Similar depots have since been opened in Yonkers, N.Y., Chicago, and Rochester, N.Y. In all these depots the milk was pasteurized at first, but in the Rochester depots, which are municipal institutions, it has been found possible by the observance of strict cleanliness in milking and storage to dispense with any process of sterilization and pasteurization and the milk is supplied raw. A fuller account of the Rochester methods, which are of the greatest interest, is given later.

The first British depot was opened on August 8th, 1899, by the St Helens Corporation on the initiative of Dr F. Drew Harris, the Medical Officer of Health². Depots were opened by Liverpool, Ashton-under-Lyne, and Dukinfield in 1901, Battersea in 1902, and Leith and Bradford in 1903. All these are municipal institutions, but the first private depot in this country was established in York in 1903 by the York Health and Housing Association at the instance of Mr Seebohm Rowntree. In Liverpool the work is conducted on a large scale. There are two sterilizing stations, and during the two and a half years ending December 31st, 1903, no less than 6295 children had been fed on the milk.

As the English milk depots differ in some respects from the French Gouttes de Lait and from the American depots it may be useful to describe the working of one of the former institutions in some detail, and for this purpose the writer has selected the Battersea depot as being the one with which he is most familiar. It may be taken as a fairly typical Infants' Milk Depot. The following description applies to the methods adopted at the present time, which are the results of nearly two years' experience.

One of the most important considerations to be borne in mind in establishing a Milk Depot is the control of the source of the milk supply. It is most important that the milk should arrive at the depot as free as possible from bacterial contamination, otherwise considerable difficulty

¹ Freeman, The Reduction in the Infantile Mortality in the City of New York, *Medical News*, New York, Sept. 5th, 1903.

² *The British Medical Journal* of August 18th, 1900, contains an interesting article by Dr Drew Harris on the St Helens Depot.

will be experienced during the hot weather and the best results will not be secured. The farm from which the milk is supplied to the Battersea depot was selected by the Medical Officer of Health from a number of farms offered by the contractor for selection. The cows are milked in the open air and never enter a shed except during a few weeks in winter. The milk is cooled down to 40° F. shortly after it is drawn from the cow and travels to the depot in sealed churns. The following is a list of the conditions accepted by the contractor.

1. The Contractor shall supply milk which must contain not less than 3.25 per cent. of butter fat and 8.75 per cent. solids not fat, and cream which must contain not less than 50 per cent. of butter fat; and the milk and cream must be free from chemical preservatives or colouring matter and be drawn from healthy cows only.

2. The Contractor shall deliver the milk and cream in sealed churns or cans to the Council's Depot, No. 28 York Road, before 8 a.m. every morning, Sunday excepted.

3. The Contractor must be prepared to increase or diminish the supply within reasonable limits, at one day's notice from the Medical Officer of Health.

4. A warranty ticket must be attached to every churn and can guaranteeing its contents to be in accordance with the specification.

5. No charge is to be made for the use of churns or cans, which when empty are to be removed by the Contractor from the Council's Depot free of charge.

6. All pails, strainers, railway churns, refrigerators, fittings and other vessels and implements brought into contact with the milk shall be thoroughly inspected before being used, be properly cleansed, scalded and dried immediately after being used, and exposed to the air in a clean place, without lids or covers.

7. The farms, water supply, drainage system, farm buildings, dairy and cattle shall be open at any reasonable time to the inspection of the Council's Medical Officer of Health, or any person duly authorised by him, and no milk or cream shall be supplied from any farm which has been certified by the said Medical Officer of Health to be in an insanitary condition.

8. The cows shall be subject, if required, to a periodical veterinary inspection by a Veterinary Surgeon duly appointed by the Council, and the Contractor shall undertake not to supply milk or cream from any cow which is diseased, newly calved or under physic.

9. The cows shall during summer be pastured, and during winter so fed that no taint is imparted to the milk. The Contractor undertakes to use no brewer's grains (wet or dry), turnip-tops or vetches.

10. The udders of the cows shall be carefully cleansed before milking, and the utmost possible cleanliness observed at every point connected with the cows, cowhouse, utensils and attendants.

11. The milk shall be carefully strained and cooled to at least 56 degrees F., immediately after milking, over a Lawrence, or other cooler of approved design, and shall be delivered at the Council's Milk Depot at a temperature not higher than 56° F.

12. The Contractor shall undertake that the refrigerators and the in-flow and

out-flow pipes, &c., are examined daily in order to see that everything is in thorough repair, and that there is no leakage.

13. No milk or cream shall be supplied from any farm on which there is a case of infectious disease.

14. The Council shall have the right to take samples of the milk or cream at any time, either at the farm or at any point in course of delivery.

15. From June to September inclusive, the Contractor when required by the Medical Officer of Health, shall pasteurise the milk before delivery.

16. Should any breach of any one or more of these clauses be at any time proved, the Contractor shall pay to the Council the sum of twenty pounds, or any lesser sum the Council may think fit, as and for liquidated damages, for each and every time such breach of any clause shall have been committed, and the Council shall deduct the same from any amount which may be due to the Contractor.

The Battersea depot is a three-storied building which was adapted for the work. The two upper stories are occupied as a residence by the manageress and some of the staff, and the work is carried on in four rooms on the ground-floor. The front room is used as a shop, in the next room the babies are weighed, the third is the bottle-washing room, and in the fourth the processes of modifying, bottling and sterilizing are carried out. The appliances in use are as follows:

- 1 One H.P. boiler.
- 1 Sterilizing chamber with trolley, as shown in the illustration.
- 2 Bottle-filling machines.
- 1 Cold storage chamber.
- 1 Cooling tank.
- 1 Electric motor to which are attached two revolving brushes for bottle-washing.
- 3 Soak tanks for dirty bottles.
- 3 Sets of rinsing jets.
- 1 Milk strainer.
- Drawing racks, churns, cans, measures, wire baskets, graduated 7 oz. bottles, etc.

The amount expended in alterations to premises and in appliances since the depot was opened in June, 1902, is about £700. The milk arrives at the depot before 6 a.m., and is at once modified by the addition of water, cream, lactose and a little salt.

The following table gives the dilutions and the amounts, given at different ages¹.

¹ The dilutions and amounts are based on the table in the leaflet, *How to bring up Children*, issued by the Medical Committee of the Hospital for Sick Children, Great Ormond Street.

Age of child	Modification	No. of bottles per day	Amount per bottle	Amount per day
During 1st fortnight	Milk 1 part, water 2 parts	9	1½ oz.	13½ oz.
„ 2nd „	„ „	9	2½ „	22½ „
„ 2nd month	„ „	9	2½ „	22½ „
„ 3rd „	Milk 1 part, water 1 part	9	3 „	27 „
„ 4th „	„ „	8	4 „	32 „
„ 5th „	„ „	7	5 „	35 „
„ 6th „	Milk 2 parts, water 1 part	7	5 „	35 „
„ 7th „	„ „	6	6 „	36 „
„ 8th „	Milk practically unmodified	6	6 „	36 „
Over 8 months	„ „	6	7 „	42 „

Cream and sugar are added so as to bring the proportion of fat and sugar to about 3·2 and 6%, respectively. The milk is varied to suit individual cases upon the request of a medical man. After modification the milk is bottled and is then, the stoppers being closed, placed in the sterilizing chamber. Steam is injected and the temperature raised to 212° F., where it remains for about 10 minutes. The bottles are then taken out of the sterilizer and rapidly cooled in the cooling-tank.

The bottles are supplied in wire baskets, each basket holding from 6 to 9 bottles and containing a 24 hours' supply. The next day the basket of empty bottles is returned and a fresh supply obtained. When a child is entered at the depot the mother is instructed by the manageress as to the proper method of using the milk and she receives the following printed leaflet.

Instructions for the use of Humanised Milk.

1. The charge for the full weekly supply of Humanised Milk for infants under six months is 1s. 6d., payable in advance. If a day's supply only is taken, the charge is 3d. The charge for the full weekly supply for infants aged from six to twelve months is 2s., or 4d. per day. Children above one year old will be charged 2s. 6d. per week, or 5d. per day. The scale of charges for children living outside the Borough is as follows:—

Under six months	2s. 3d. per week, or 4d. per day.
Six months to twelve months	...	2s. 9d.	„ 5d. „
Over one year old	...	3s. 3d.	„ 6d. „

2. The Depot is open from 11 a.m. to 6 p.m. on week-days, and is closed on Sundays.

3. The milk will be supplied in bottles in a basket, each bottle containing sufficient milk for one meal, the amount varying with the age of the child. Infants under two months receive nine bottles per day; older children receive fewer bottles, as they should be fed less frequently.

4. If children are sent for the milk, they must be warned not to tamper with the stoppers of the bottles. On no account must a bottle be opened until the infant is ready to be fed.

5. Just before using, each bottle should be placed unopened in a basin, or jug of hot water, and warmed to the proper temperature. The bottle should then be opened and the teat put on. The child should be fed at regular intervals, and fed from these bottles only. On no account should any other feeding-bottle be used. The teat should be kept scrupulously clean.

6. When all the milk in one bottle is not used, the remainder must not be warmed up again, but a fresh bottle opened for the next meal. Where there are other children this milk need not be wasted.

7. On no account should any other food be given unless ordered by a doctor.

8. After using, the bottles should be thoroughly rinsed in cold water.

9. Breakages will be charged for at the rate of 1*d.* per bottle, and damage to baskets must be made good. All bottles, baskets, and rubber rings not returned to the Depot will be charged full value.

10. It is important that the child should be brought once a week to be weighed. The Depot is open for this purpose on Tuesdays and Wednesdays from 2.30 to 4 p.m.

11. The presence of Infectious Disease in a house must be at once notified to the Medical Officer of Health.

N.B.—*The milk should never be used in preference to mothers' milk, which is the best of all foods for young infants.*

It will be seen that this method of infant feeding is a very simple matter as far as the mother is concerned. When feeding time arrives all she has to do is to place the bottle, unopened, in a basin of warm water until it reaches body temperature, to open the bottle, put on a rubber teat supplied at the depot and feed the baby from the sterilized bottle direct. There is no need for a "feeding bottle," which alone is a great advantage.

The homes of the children fed on the milk are visited by the lady sanitary inspectors, who endeavour to secure that the instructions are properly carried out. If the child does not appear to be progressing favourably the mothers are strongly advised to seek medical advice, and if the child has been using the milk for more than two or three weeks and is not under medical supervision the mother is advised to give a little gravy or raw meat juice in addition to the milk, and written instructions for the preparation of raw meat juice are given to her. Mothers are urged to bring the children once a week to be weighed, but it has been found impossible to insist on this. There appears to be a prejudice against baby-weighing, at all events the number of children who are brought to the depot for this purpose is small.

Arrangements have been made with the Board of Guardians by

which the relieving officers are empowered to issue orders on the depot in lieu of giving money in outdoor relief. Similar arrangements have been made with various local charitable organizations.

There are several points of difference in the administration of the depots in England and America and on the Continent. One point is the treatment of the milk. In many of the French depots the milk is sterilized, but not modified, except for very young infants, and the process of sterilization consists of keeping the milk at 102° C. for from 45 to 60 minutes. At Fécamp, however, the milk is diluted with water in the proportion of one part to two parts of milk, and the same modification is adopted at St Helens, where the milk is sterilized by the French method. In the American depots the milk is pasteurized, with or without modification, except at Rochester, where it is supplied modified, but raw. The preparations and quantities at Bradford and York are similar to those at Battersea, which have already been given, and the milk is kept at 212° F. for from 10 to 20 minutes.

In the depot at Earle Road, Liverpool, which is the most complete in this country, the milk is kept at a temperature of 210° F. for from 20 to 30 minutes, and the preparations are as given below¹.

Age	Quantity of pure milk for 24 hours in ounces	Water in ounces
1—2 weeks	6 $\frac{3}{4}$	6 $\frac{3}{4}$
2—8 „	13 $\frac{1}{2}$	13 $\frac{1}{2}$
2—3 months	20 $\frac{3}{4}$	10 $\frac{1}{2}$
3—5 „	30	15
5—7 „	36	12
Over 7 months	36	12

2 $\frac{1}{2}$ ounces of cream, 1 $\frac{1}{2}$ ounces of sugar, and $\frac{1}{2}$ ounce of salt, to be added to each gallon of mixture.

The most important difference, however, between the English and American depots and the French Gouttes de Lait is in the supervision of the children. In most of the French institutions the supply is stopped if the child is not brought to the depot regularly to be weighed and examined by a medical man, but in the English and American depots there is no hard and fast rule of this kind, and such supervision as is exercised by the officers of the depot is carried out by nurses or lady inspectors acting under the instructions of the medical director of the institution, who in England is the Medical Officer of Health.

¹ Mussen, Supply of Sterilized Humanized Milk for Infants, *Journal of State Medicine*, Oct. 1903.

As a matter of fact most of the children come to the depot on the advice of a doctor and begin the milk under medical supervision, and should the progress of the child at any time be unsatisfactory, a fact which the parents are by no means reluctant to bring to the notice of the management, the mother is strongly advised to take the advice of her own doctor. In the English depots the object is to secure as far as possible the supervision of the regular medical attendant rather than to appoint a municipal doctor to give gratuitous advice. It is questionable whether the latter plan would meet with the approval of the medical profession in this country, as it is easy to see that it might lead to an objectionable form of "hospital abuse."

Objects of the Infants' Milk Depot.

So much having been said on the history and practical working of the Infants' Milk Depot it may now be asked upon what grounds can the existence of these institutions be justified? For what object were they established, and is there any evidence that the object has been attained? The object of the Infants' Milk Depot is, as stated by Dufour, "lutter contre l'excessive mortalité des enfants de la ville élevés artificiellement, surtout dans la classe pauvre¹," and it may be well to consider in some detail the grounds on which it is held that the depot is capable of achieving this object. Although much yet remains to be said on the subject of infantile mortality there can be no doubt that the most important condition giving rise to the deplorable waste of infant life which year by year recurs in our urban centres is defective infant feeding. It is unnecessary to recapitulate the evidence for this statement: it will hardly be disputed, but it may be pointed out that the mortality due to defective feeding cannot be fully estimated from the deaths from the diarrhoeal and digestive diseases. The impaired nutrition resulting from defective feeding must render the child specially liable to succumb to acute disease and to the various forms of tuberculosis.

Breast-feeding.

Breast-feeding is undoubtedly the best method of infant feeding, but there is reason to think that breast-feeding is becoming increasingly difficult to secure. The writer is not in possession of

¹ Dufour, *Comment on crée une Goutte de Lait*.

any statistics which support this view, but he knows no practitioner of long experience who does not agree to it. There is a tendency to assume that hand-feeding is a phenomenon entirely or almost entirely due to social and economic conditions and to neglect possible physiological factors. This is another point on which statistical evidence is lacking, but the writer inclines to the opinion, which is based on his own experience and that of more experienced practitioners, that a considerable number of mothers who wish to suckle their children are unable from lack of milk to do so. But whatever the causes of hand-feeding may be, and whether it is or is not increasing, there can be no doubt that the practice is deeply rooted in our social life. Doubtless breast-feeding could be increased by the growth of a more healthy public opinion on this question, by special factory legislation, and by the establishment of Consultations de Nourrissons on Budin's methods, but it is to be feared that in the absence of something like a revolution in our social and economic organization hand-feeding will continue to be so prevalent as to make the difference between good and bad hand-feeding a matter of immense importance.

The problem of finding a satisfactory substitute for mother's milk is one of great difficulty, and it may be questioned whether it will ever be completely solved. In the meantime thousands of mothers have to feed their children as best they can, and while our knowledge of artificial infant feeding is so defective that physicians of the highest eminence hold the most diverse opinions on such subjects as the relation of sterilized milk to infantile scurvy and the desirability of modifying cows' milk for infants, it is not surprising that the ordinary housewife should fail to achieve satisfactory results. Amongst the poor the grossest mistakes are made: young infants are not infrequently given such articles as hard-boiled eggs, cheese, carrots, beer, and even spirits, and other items of the comprehensive dietary known as "what we have ourselves." Assuming, however, that the mother has sufficient intelligence to avoid such lethal dietetic errors she has three classes of food from which to select a substitute for human milk, viz., proprietary foods, condensed milk, and cows' milk more or less modified and artificialized.

In seeking for a substitute for mother's milk it should be borne in mind, as Chapin points out, that "anything aside from breast-milk that is put into an infant's stomach is a foreign substance that may cause digestive disturbance¹," and the aim should be to obtain a food

¹ Chapin, *The Theory and Practice of Infant Feeding*, 1902.

resembling as closely as possible human milk. Tried by this standard proprietary foods are at once put out of court. Milk is a purely animal product, while proprietary foods are largely vegetable in composition as nearly all contain wheat flour or other matter of vegetable origin, and many contain unaltered starch, a substance the young infant is quite unable to digest. Generally speaking these foods are deficient in fat, too rich in carbohydrate, and contain no anti-scorbutic element. They are not now in favour with the profession, and few will dispute Still's opinion that "There are few cases in which any of these proprietary foods should be allowed to become the principal article of diet."

Condensed Milk.

Condensed milk is a popular infant food. It appears to be cheap, is easy to prepare, and is not infrequently recommended by medical men as being "safer" than cows' milk in hot weather.

In nutritive properties condensed milk is seriously deficient. Apart from the separated condensed milks which are destitute of fat it may be said that most of the brands of condensed milk upon the market are deficient in fat, and contain an excessive amount of cane-sugar, while all lack the anti-scorbutic elements. There is abundant clinical evidence to associate condensed milk and rickets as cause and effect.

The popular idea that condensed milk is a comparatively "safe" food during the diarrhoea season is not in accord with fact. Newsholme found that in 191 cases of fatal diarrhoea in Brighton in the three years 1900-02¹ the method of feeding was as follows:—

Breast	9.4 %
Cows' milk	46.6 „
Condensed milk	44 „

Meredith Richards² investigated 183 deaths from diarrhoea in infants under six months in Croydon during the same years, and found the method of feeding to be as follows:—

Breast	14 %
Cows' milk	48 „
Condensed milk	33 „

¹ *Annual Report on the Health of Brighton*, 1902.

² *This Journal*, 1903.

Of course, these figures cannot be taken as showing the relative incidence or fatality of diarrhoea in infants fed on cows' milk and condensed milk respectively, as the number of infants comprised in each of these two classes is not stated, but it is improbable that more than 44% of the total infants in Brighton were fed on condensed milk. There is, therefore, no reason to think that the infants fed on condensed milk are more secure from diarrhoea than those fed on cows' milk. Nor is this surprising. When the tin is opened condensed milk is as liable to contamination as cows' milk, especially as the tin contains sufficient material to last two or three days. Moreover, condensed milk is not necessarily sterile. During the years 1900-02 75 samples of condensed milk were examined by the Bacteriologist of the Liverpool Health Department, and the majority were found to be not sterile. Dr Hope says, "Bacteria are usually present, and the milk which was originally condensed might have contained various products of the decomposition of bacteria. These products are masked subsequently by the large quantity of sugar present, but their irritant properties are not destroyed¹."

Methods of feeding are, however, of importance in connection with diseases other than diarrhoea. There is need for more knowledge on this subject. What is wanted is an investigation, clinical, pathological and bacteriological, into the various methods of artificial feeding and their influence on nutrition. We want to know more of the influence of various foods on the child's powers of resistance to disease in general and to certain diseases in particular. It is probable that fuller knowledge would reveal further serious disadvantages to the use of patent foods and condensed milks. Park and Holt have lately conducted an enquiry somewhat on these lines in the tenement-houses of New York City, and it is interesting to note that the results with condensed milk in the summer observations were particularly unsatisfactory. "These children were often apparently in good condition until attacked with acute disease, when they offered but little resistance and seemed to succumb more quickly than any other class of patients²."

Niven³ has compiled the following interesting table in connection

¹ *Annual Report on the Health of Liverpool*, 1902.

² Park and Holt, Report upon the results with different kinds of pure and impure milk in infant feeding in tenement-houses and institutions of New York City, *Medical News*, New York, Dec. 5, 1903.

³ Quoted by T. D. Lister, *Infant Feeding and Milk Supply*, 1903.

with the question of the relation of condensed milk to infantile mortality.

Two Districts of Manchester, showing the Comparative Feeding of 533 Children.

Method of feeding	Ancoats (433)	Chorlton-upon-Medlock (100)
Breast-fed	85·9	81·0
Artificially fed	14·1	19·0
	100·0	100·0
Of the artificially fed:		
Cows' milk	62·0	89·0
Condensed milks	38·0	11·0
	100·0	100·0
Infantile mortality	234	184

This table shows that although rather more children were breast-fed in Ancoats than in Chorlton-upon-Medlock, the use of condensed milk was very much more common in the former district and was associated with a far higher rate of infantile mortality. It is not, of course, claimed that these figures are conclusive, but they are certainly suggestive and indicate a line of enquiry which might usefully be followed further.

Cows' Milk.

We now come to the consideration of cows' milk, which is generally agreed by the profession to be the best substitute for human milk generally available. But though cows' milk resembles human milk much more closely than patent foods or condensed milk, there are important points of difference apart from the question of contamination bacterial or otherwise. Cows' milk has a hard curd, and is adapted for the digestion of an animal with four stomachs. The human infant has but one stomach, and that is adapted for the reception of a milk with a soft curd.

The chemical differences are as great as the mechanical. The importance of these differences is, however, a matter of dispute. Budin and the French physicians give sterilized milk unmodified,

even to very young infants. In the Havre Goutte de Lait the milk is entirely unmodified. Some writers, *e.g.* Variot, affirm that it is modification and not sterilization that gives rise to infantile scurvy. On the other hand, Rotch and the Americans, who have done much to place infant feeding on a more scientific basis, insist on the importance, of that individualized modification,—percentage feeding. The weight of opinion in this country seems to be with the American rather than the French physicians, and in the present state of our knowledge it would appear to be safer to employ some form of modification, although the American refinements are perhaps unnecessary for babies on this side the Atlantic.

The most important point of difference, however, between human milk and cows' milk is that whereas the former passes from the secreting gland direct to the baby's mouth and is practically sterile, cows' milk in making the journey between these two points is seriously exposed to contamination. Contamination by chemical preservatives, though not so frequent as formerly, is still far too common, but it is almost insignificant compared with the importance of bacterial contamination.

Bacterial Contamination.

The process of milking as carried on in this country has been happily described by Dr Leslie Mackenzie as a "process of unscientific inoculation of a pure or almost pure medium with unknown quantities of unspecified germs¹." From the time it is drawn from the ungroomed filthy cows until by means of the long-tubed bottle it reaches the mouth of the baby, cows' milk is continually exposed to serious pollution, and gross bacterial fouling is so common as to be almost universal. The influence of bacteria-polluted milk on health is far from being fully understood, but it may be assumed that clean milk is preferable to dirty milk. We know, however, that milk has often acted as a carrier of the infections of enteric fever, scarlet fever, diphtheria, cholera and tuberculosis, although its influence on the latter disease has possibly been exaggerated, and there is now a mass of evidence that certain forms of sore-throat are closely associated with the consumption of milk from diseased cows. It is most probable that our list of milk-borne diseases is far from complete, and that fuller knowledge will add to the dangers we now know to exist in the present state of the milk supply.

¹ Mackenzie, *Edinburgh Journal of Medicine*, 1899.

The question of the chief place of contamination is important. In the case of the diseases already mentioned it is usually the farm, but in diarrhoea, the most fatal of the milk-borne diseases, contamination probably takes place chiefly in the home of the consumer. This view, however, is disputed by Delépine, who, from the examination of a large number of milk samples during an investigation extending over seven years, has arrived at the conclusion that there is a distinct relationship between epidemic diarrhoea and food poisoning, that both are due to the infection of the food by members of the colon group of bacilli derived from faecal contamination, and that the contamination is chiefly derived from the farm.

"My results do not exclude infection at the home of the consumer, or during transit from the farm, but they indicate that infection at the farm, or through vessels infected at the farm and used by the farmer for the storage and carriage of milk must be of paramount importance. None of ~~the~~ milk I have examined had been exposed to any influence attributable to a consumer's home. It will be noticed that a large proportion of the samples of milk obtained from cans at railway stations or at the farms is already infectious before it reaches the consumer; also the degree of noxiousness acquired through infection is proportional to the length of time the milk has been kept, and the temperature which it has been exposed to, before it reaches the consumer¹."

Newsholme² does not accept this view. He does not deny that serious epidemics of diarrhoea occur which are due to the contamination of a single milk supply, and that such epidemics are analogous to milk outbreaks of enteric fever, but he holds that "the ordinary sporadic cases of diarrhoea are due to domestic infection of milk or other foods, or to the direct swallowing of infectious dust." From an investigation of the different supplies of milk among the families in Brighton invaded by diarrhoea in the years 1900—02 he found no evidence of special incidence on single milk supplies. In the 89 fatal cases of diarrhoea fed on cows' milk there were no less than 41 sources of milk supply, and he concludes that "Unless we assume that a very large proportion of non-fatal cases occurred, we must infer, in view of the diffusibility of milk, that most of these milk supplies were non-infective before they reached the home of the individual patient." Moreover 9·4 %

¹ This *Journal*, 1903.

² The importance of the home contamination of food in the causation of diarrhoea was first pointed out by Newsholme in his Presidential Address to the Incorporated Society of Medical Officers of Health, 1899. See *Public Health*, December 1899.

of Newsholme's total 181 cases were breast-fed, and 44% fed on condensed milk¹.

Further evidence in support of Newsholme's view has been brought forward by Meredith Richards². The latter writer examined the records of 22 cases of food poisoning, excluding cases due to tinned food, and found a striking difference between the seasonal incidence of these outbreaks and that of fatal diarrhoea. He also points out that Chesterfield with a diarrhoeal rate of 54.4 per 1000 births during the years 1896—99, derived 98% of its whole milk supply from cow-sheds situated within the borough or within a radius of five miles, and that out of 253 fatal cases of diarrhoea occurring in infants in Croydon over 12% were breast-fed, while only a little more than half received fresh cows' milk³. The investigations of Park and Holt in New York lend no support to Delépine's view. The ten physicians engaged in this enquiry were agreed that the most important factor was intelligent care, while "most of the physicians stated that, leaving out the very worst store milk in summer, the results were very much less affected by the character of the milk than they had anticipated, and distinctly less than by the sort of care the infants received."

On the whole, it may be said that weight of evidence is distinctly in favour of the view that in fatal diarrhoea contamination of the food takes place chiefly in the home of the consumer.

From the foregoing considerations we may conclude that cows' milk is the best substitute for human milk generally available, that it is probably better to modify it for the use of young infants, and that it is most important to protect it against contamination, especially against contamination within the home of the consumer.

But even the purest cows' milk of the most nicely adjusted percentage composition may be a source of danger in the hands of a

¹ Newsholme, Remarks on the Causation of Epidemic Diarrhoea, *Transactions of the Epidemiological Society*, Vol. xxii. N. S. p. 34. See also *Annual Report on the Health of Brighton*, 1902.

² *This Journal*, 1903.

³ The relation of condensed milk to diarrhoea requires further investigation. Delépine and Hope have shown that condensed milk is frequently non-sterile, and it does not follow that condensed milk which is sterile, *i.e.* containing no living bacteria, is necessarily non-toxic. Moreover, further information is required as to the relation of attack-rate to death-rate in infants fed on condensed milk and other foods. The case mortality in infants whose nutrition has been impaired by a condensed milk diet may be exceptionally heavy, as would appear from the enquiries of Park and Holt, see p. 344. If this be so, a comparatively small number of tins of condensed milk containing infective material may give rise to a disproportionately large number of deaths.

careless or ignorant mother. The question of quantity as well as quality has to be borne in mind. The child may be over-fed, either by being fed too frequently or by being given an excessive amount at each meal. Both forms of over-feeding are common and give rise to serious consequences. Budin attaches great importance to "suralimentation" as a factor in the production of gastro-intestinal disorders, and he lays stress on the advantages of supplying the day's milk in separate bottles, each bottle containing the proper quantity for one meal¹. By this method of supply, which is employed in most of the French Gouttes de Lait and in all the British Milk Depots, the danger of over-feeding is considerably lessened, as both the number of meals and the proper quantity at each meal are clearly indicated. The Infants' Milk Depot, however, has other important advantages. The milk supplied is free from chemical preservatives—this alone is an important consideration—and, as in many depots, if not in all, care is taken to supply milk produced under clean conditions, the original bacterial content is probably much less than that of the milk generally supplied in the district. By the process of sterilization the initial pollution is wholly, or almost wholly, neutralized², and as each meal is supplied in a separate bottle the possibilities of home contamination are reduced to a minimum. Moreover, the milk is supplied at a price which is not beyond the reach of the poor. If then the work of the depot is under the supervision of a medical man and care be taken not to discourage breast-feeding, it would appear that from the general principles of preventive medicine there is ample justification for the existence of these institutions.

Results.

We may now examine the results of these institutions so far as they can be ascertained at the present time. A considerable amount of statistical matter has been issued in respect of the various depots in Europe and America, but after a careful consideration of the numerous documents he has been able to consult, the writer has arrived at the conclusion that it is doubtful whether the value of these institutions can be expressed in figures. But the Infants' Milk Depot is not the only preventive measure of which this can be said. There

¹ Budin, *Le Nourrisson*, Paris, 1903.

² Sterilization is not an essential part of the work of an Infants' Milk Depot. In the depots at Rochester, U. S. A., it has been found possible by the employment of aseptic methods to supply the milk raw. This is of course the ideal method.

are indeed few measures of public health administration whose beneficial effect can be demonstrated inductively by statistics, as can be done in the case of vaccination. The conditions determining the prevalence and fatality of a particular disease or group of diseases are so exceedingly numerous and interdependent that it is almost impossible to trace the effects of any one cause. For instance, the precise effect of hospital isolation on the prevalence and fatality of any of the chief infectious diseases is still a matter of controversy, although a considerable mass of material is available for analysis. To trace the effect of a milk depot is a much more difficult problem. From the statistical standpoint a depot is analogous to a hospital for sick children. It would be an extremely difficult task to estimate in figures the influence on child mortality of the various children's hospitals in London; and in the case of the milk depot the difficulty is even greater, as the few depots which exist at the present time have been but recently established, and in every case the operations have been conducted on a comparatively small scale. In this, as in most medical questions, clinical testimony is of far greater value than statistics, and this testimony is almost uniformly favourable to the depots. In Battersea this is certainly the case. In February, 1904, the writer addressed a circular letter to the medical practitioners in the district and to the visiting physicians of certain children's hospitals to which Battersea children are taken, asking for an expression of opinion as to the value of the milk. Replies were received from 44 practitioners, three of whom refrained from expressing any opinion on the ground that they had not had sufficient data. The remaining replies, with one exception, were favourable, in most cases highly so; only one practitioner expressed himself as disappointed with the results obtained from the use of the milk. The testimony in favour of the Straus depots of the observers who conducted what was probably the most methodical and extensive investigation into infant feeding yet made is quoted on page 361.

Statistical Evidence.

The statistical evidence it must be confessed is uncertain. All the methods which have yet been employed are beset with fallacies, which in no case appear to have been successfully avoided.

The method usually adopted is to compare the death-rate amongst the children fed from the institution with the infantile mortality in the

town. Budin recently presented a report to the Académie de Médecine, Paris, on the work of his Consultation de Nourrissons at the Clinique Tarnier. From the account of this report in the *British Medical Journal* of February 20th, 1904, it appears that since March, 1898, 712 children have attended the Consultation from birth for periods varying from less than one month to two years, and that 26 died; a proportion of 36·5 per 1000. As the average period of attendance was nine and a half months, the annual mortality rate is estimated at 46 per 1000. For comparative purposes it is stated that in Paris during 1898, 1899, 1900 there were 20,282 deaths in 113,805 infants under one year, deducting the infants sent to nurse outside the city, or a proportion of 178 per 1000—a figure which compares most unfavourably with the mortality rate at the Clinique. But these figures are not really comparable, for while the rate in the city is a death-rate of infants under one year, no less than 243 of the 712 children at the Clinique attended during their second year—a period when the mortality rate is distinctly lower than in the first year. It would be better to either exclude these 243 children or compare the rate 46 per 1000 with the death-rate in Paris of children under *two* years¹.

In the comparative figures calculated for the St Helens Depot by Drew Harris this error appears to have been avoided, and the results calculated in the number of children under one year. The figures are as follows²:

Year	No. of children on books	Death-rate per 1000 among children at depot	Infantile death-rate borough of St Helens
1899	232	103	157
1900	332	102	188
1901	282	106	175
1902	200	82	167

In the first three years of this table the children fed on the milk for less than one week were deducted, as in these cases the milk was not given a fair trial; in 1902 the children fed for less than 14 days were deducted.

¹ The difference between the two rates, however, is too great to be explained by the difference in age-periods. There can be no doubt that the children at the Clinique had a much better chance of survival than children living under average conditions in Paris, although this cannot be expressed numerically from the data given. The 712 children were all born in the institution and all were under highly skilled medical supervision.

² *Annual Report on the Health of St Helens, 1902.*

Hope¹ has recently calculated the death-rate of the children attending the Liverpool depots. The total number of children on the books up to December 31st, 1903, was 6295, but in many cases it was found impossible to obtain sufficient information. In the 4453 cases kept under close observation there was a mortality rate of 78 per 1000—a rate which compares favourably with the infantile mortality rate in the city, which, in the three years under consideration, was as follows :

Year	Infantile mortality
1901	188
1902	163
1903	151
<hr/>	
Average	167·3

Adopting the same method of comparison, the writer calculated the mortality rate in the infants attending the Battersea depot during the six months ending December 31st, 1902². During this period there were 466 children who were kept under observation. Deducting 72 children over one year there remained 394 infants under one year, of whom 39 died. This gives a mortality rate of 98·9 per 1000, the infantile mortality in the borough during the same period being 143 per 1000. In 14 of the 39 fatal cases the milk had been taken for less than one week, and when these cases were deducted and the deaths during the first week of life in the borough also deducted, the rates in the depot and borough respectively were 63·4 and 118·9. Similar rates have been calculated for several of the Gouttes de Lait, the results in all cases being highly favourable to the institution.

Further reflection has convinced the writer that it is most difficult to assess the value of the results obtained by the use of this comparative method. The method itself is so beset with fallacies that any conclusions based upon it must be received with caution. In the first place it is a mistake in this connection to treat the first year of life as one age-period. It is really a collection of age-periods, the earlier of which differ from the later in mortality rates far more than the whole first year does from any subsequent period. From the infantile life-tables in the 54th Annual Report of the Registrar-General it appears that "the mortality is highest in the first day of life, and then falls rapidly, though still high

¹ "Infantile Mortality and the supply of Humanised Sterilised Milk," a paper read before the Liverpool Medical Institution on March 17, and reported in the *British Medical Journal*, March 26th, 1904.

² *Annual Report on the Health of Battersea*, 1902.

in the remaining days of the first week. The mortality falls enormously in the second week, remains at nearly the same level through the third, and shows a considerable decline in the fourth week. In the second month the mortality is only a small fraction of that in the first month; it then falls more gradually to the end of the 7th or 8th month, after which but little change occurs¹. If, then, the depot children contain a greater or less proportion of infants in the first few weeks of life than the children in the town, the comparison is at once vitiated, for the age-periods are not the same. The real mortality rate can only be calculated from an infantile life-table. Moreover, a year is too long a period on which to base the mortality rate. The population attending the depot is most numerous during the season of greatest risk, namely the third quarter of the year, and the number of deaths, therefore, especially the deaths from diarrhoea, will be disproportionately high. It would be more satisfactory to calculate a separate rate for each quarter. Another difficulty arises from the varying periods during which the children remain on the milk. Many come to the depot upon medical advice while suffering from some disorder, and when recovery takes place the milk is discontinued. To include infants who have attended the depot for not more than a week or two in the depot population, in calculating the ordinary annual mortality rate per 1000, is distinctly unfair to the institution. A rate should be calculated on a group of children of the same age who have attended the depot during the whole period upon which the rate is based.

But assuming the technical statistical difficulties to be overcome, it is doubtful whether we are in a position to form a definite opinion from the results of this method. It may be urged that the mother who will take the trouble to send regularly every day for the milk may be assumed to be more than usually mindful of the welfare of her children, who would, therefore, in any case have had a better chance of survival. To some extent this is true, although the depot method of feeding saves so much labour that it might be expected to attract the less industrious housewives. But there are considerations on the other side. In comparing the institution infants with those in the town, we are comparing a class entirely hand-fed with one containing a very large number of breast-fed infants, and it is not contended that the depot milk is anything but an imperfect substitute for mother's milk. And while nearly all the depot children are drawn from the poorer classes

¹ Newsholme, *Vital Statistics*.

living in the least healthy districts, the town population includes the middle and upper classes amongst whom the infantile mortality is relatively low. More important still is the fact that many of the children begin to use the milk when they are already more or less seriously ill. No less than 50 per cent. of the children attending the Liverpool depots¹ were ill at the beginning of their attendance. The depot death-rate is therefore somewhat analogous to a dispensary death-rate, and the children cannot in this respect be compared with an ordinary child population. It may be that these opposing considerations neutralize each other. The writer's opinion is that the balance is distinctly in favour of the depots, and that the figures quoted above under-estimate rather than over-estimate the value of these institutions. But from a purely statistical standpoint this conclusion cannot be drawn from the figures as they stand. The only way of applying this method so that conclusive results might be expected, would be to calculate by an infantile life-table a death-rate in each quarter for children attending the depot during the whole period of observation, and for hand-fed children of the same class, living in the same district under similar conditions, and exposed to the same influences. It does not appear that such statistics have yet been published.

Another method is to compare the infantile mortality in the town before and after the opening of the depot. This method is employed by Dr Peyroux of Elbeuf in his attacks on the Gouttes de Lait². Taking the nine French towns in which the oldest and most important of these institutions have been established, he has ascertained the proportion of the deaths under one year to the deaths at all ages, and also the proportion per 1000 births, before and after the establishment of the Goutte de Lait. The method of estimating infantile mortality by the proportion of deaths under one year to total deaths is so fallacious that it is surprising that it should have been seriously put forward. Without considering results based on this method we may at once turn to those calculated on the proportion of deaths under one year per 1000 births. Dr Peyroux' figures, which are compiled from the official returns, may be arranged in tabular form as follows³:

¹ Hope, *op. cit.*

² Peyroux, Consultations de Nourrissons et Gouttes de Lait, *La Semaine Médicale*, Paris, December 24th, 1902.

³ In considering these figures it should be borne in mind that still-births are registered in France.

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Town	Before the Goutte de Lait		After the Goutte de Lait	
	Years	Deaths under 1 year per 1000 births	Years	Deaths under 1 year per 1000 births
Fécamp	1881—94	214	1895—1900	194
Grenoble	1891—94	168	1895—1901	141
Bourg	1891—98	141	1899—1901	130
Elbeuf	1891—98	284	1899—1900	307
Havre	1891—98	207	1899—1900	219
Nantes	1891—98	182	1899—1901	140
Nancy	1891—99	183	1900—1901	177
Rouen	1891—1900	305	1901	262
Versailles	1891—1900	189	1901	180

From these figures Peyroux contends that “les résultats des Gouttes de Lait sont négatifs à Elbeuf, à Nancy, au Havre; ils sont médiocres à Bourg et à Versailles, un peu plus satisfaisants à Fécamp. Seules, les Gouttes de Lait de Rouen, de Grenoble et de Nantes paraissent avoir donné des résultats positifs.” He will not, however, allow that the results in the latter towns are really positive. He points out that at Grenoble, for instance, in 1891—1901 the mean number of births was 1357 per annum, while the yearly number of infants fed from the Goutte de Lait was only 72, most of whom were not fed from the institution more than three months, and he holds that the Goutte de Lait, therefore, could have had little effect in diminishing the infantile mortality. He concludes, therefore, that on the whole the results of the Gouttes de Lait have been mediocre, and for the reason that “très scientifiques en théorie, ces œuvres ne peuvent rien donner en pratique.”

Assuming for the moment that conclusions as to the value of the Gouttes de Lait can be drawn from these figures, it by no means follows that the results have been mediocre. If the Bourg Goutte de Lait with its small *clientèle* brought down the infantile mortality in Bourg from 141 to 130 per 1000 during three exceptionally hot summers the promoters of that institution have good cause for congratulation. It is, however, doubtful whether any conclusions can legitimately be drawn from these figures. In the first place, as Peyroux has himself indicated, the work of the institutions has been conducted on far too small a scale to produce an appreciable effect during the short period of observation. For instance, the total number of children fed from the Havre Goutte de Lait in 1899 was only 305 while the number of births in the town was 4082. In 1900 the corresponding figures were 304 and 3919¹. The same objection would

¹ Caron, *L'Eure des Gouttes de Lait*, Havre, 1903.

apply to any figures based on the movement of infantile mortality in Paris. According to Variot¹ the infantile population of Paris is about 40,000, the number of children attending the various Consultations is not more than about 800 or 900. It is obvious, as Bertillon points out², that the Gouttes de Lait in Paris are too small to produce an appreciable result.

A still more serious objection is that the period of observation is too short. To estimate the value of any measure for the protection of child life, even if conducted on a large scale, by the mortality occurring in the first few years after its adoption is obviously most untrustworthy, as infantile mortality is highly sensitive to varying meteorological conditions and to the epidemic prevalence of such diseases as whooping cough. As a matter of fact, all the Gouttes de Lait selected by Peyroux, except those at Fécamp and Grenoble, began their work either just before or during a period of exceptionally hot summers. The following table shows this clearly for the Goutte de Lait at Havre³:

Town of Havre.

Year	Deaths under 1 year per 1000 births	Deaths under 1 year from diarrhoea per 1000 births	Maximum temperature, June—September
1891	195	66	20·7° C.
1892	227	75	21·1
1893	220	117	21·4
1894	211	116	19·1
1895	215	128	22·9
1896	188	85	20·9
1897	191	115	21·9
1898	223	125	22·5
Goutte de Lait established January 1899.			
1899	212	136	24·1
1900	221	136	22·6
1901	175	94·8	24
1902	191	88·4	19

It is interesting to note that the infantile mortality was higher in 1902 than in the previous year, although the maximum temperature and the diarrhoea rate were comparatively low. This is explained by a very fatal epidemic of measles at the end of 1902.

Further evidence of the fallacies underlying the method we are now considering is afforded by the figures of some of the British milk depots

¹ Variot, L'Avenir des Gouttes de Lait, *Archives de Médecine des Enfants*, April 1903.

² Letter to the writer, March 1904.

³ Caron, *op. cit.*

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which, unlike the Gouttes de Lait, began operations before or during a period of cold, wet summers. In Liverpool, for instance, the first depot was opened in May 1901, and in the following year a second depot was established and the work greatly extended. The infantile mortality figures of the city show a great and progressive reduction in 1902-3.

Years	Deaths under 1 year per 1000 births
1896-1900	188
1901	188
1902	163
1903	151

The Battersea depot opened in June 1902, and the infantile mortality figures are as follows:

Years	Deaths under 1 year per 1000 births
1897-1901	161·8
1902	136
1903	135

The summers of 1902-3 were exceptionally cold and wet and therefore favourable to the relatively low rates of mortality which prevailed in the country generally. How far the reduction in Liverpool and Battersea was due to the weather or to the depots it is impossible to say, but there is no reason to doubt that the former was the preponderating condition.

The fallacies which occur when too short a period of observation is taken are more serious than those arising from the small number of children fed from the depot, for though the number actually may be small, the educational influence of the depot, which some consider its most important effect, may not be inconsiderable if its operations extend over a fairly long period. It will be noted that at Fécamp and Grenoble, the two oldest of the institutions mentioned by Peyroux, there was an appreciable reduction in the mortality which appears to be independent of weather conditions. Would it not be possible, then, to draw reliable conclusions from the movement of infantile mortality provided that the depot had been at work for, say, a decennium though only working on a comparatively small scale? In this connection the New York figures are of interest. The Straus Milk Charity was established in 1893 and has since steadily increased its area of operations. Compared with the enormous population of New York the number of children fed from the 14 depots may be small, but its

educational effect extending over 10 years has probably been not inconsiderable. To what extent then has the remarkable reduction in the New York infantile mortality in the last decennium been due to the Straus depots? The complexity of this problem is at once apparent from a consideration of the chart on p. 357, which was compiled by Dr Rowland G. Freeman, Mr Straus' chief medical adviser¹.

It appears that the Straus Milk Charity was not the only ameliorative influence which came into operation after 1893. Since that date the quality of the milk supplied in the city has improved, sterilization of milk has become general², many streets have been paved with asphalt, a better system of refuse removal has been introduced, open spaces have been provided, anti-toxin has come into use, the condition of the tenement-houses has been improved, and the St John's Guild and other similar organizations have been the means of providing sea-trips for many thousands of children in New York during the hot weather in July and August. How is it possible to isolate the Straus depots from these agencies and measure their influence on the decline in the infantile mortality? It would seem, then, that the method of comparing the mortality in the same town before and after the establishment of the milk depot may be unreliable even if a fairly long period of observation be taken unless a searching analysis be made of the local conditions.

The third, and perhaps the most promising, of the purely statistical methods is to compare the movement of infantile mortality in the district before and after the establishment of the depot with that in neighbouring districts containing no depot but in other respects similar. Paffenholz gives figures compiled on this method for the depot at Yonkers, N.Y., U.S.A., which was opened in 1895. The figures relate to Yonkers and three other suburbs of New York³.

¹ Freeman, *The Reduction in the Infantile Mortality in the City of New York, and the agencies which have been instrumental in bringing it about*, *Medical News*, New York, Sept. 5th, 1903.

² Sterilization of milk is now so generally practised in the tenements of New York that the investigators of the Rockefeller Institute, an account of whose work is given on pp. 359, 360, discovered that, "In the summer of 1902 especially it was rare to find an infant fed on raw milk." This perhaps has been the chief factor in the decline in the mortality.

³ Paffenholz, *Wichtige Aufgaben der öffentlichen und privaten Wohlfahrtspflege auf dem Gebiete des künstlichen Ernährungs des Säuglings*, Bonn, 1902.

Period	Hoboken 55,000 pop.		Long Island City 40,000 pop.		Newburgh 26,000 pop.		Yonkers 38,000 pop.	
June, July, August and September in each year	Deaths		Deaths		Deaths		Deaths	
	Under 5 years	From diarrhoea	Under 5 years	From diarrhoea	Under 5 years	From diarrhoea	Under 5 years	From diarrhoea
1892—95	289	104	225	90	75	30	162	91
1896	352	110	257	115	72	43	135	48
Difference	+ 63	+ 6	+ 32	+ 25	- 3	+ 13	- 27	- 43

The period of observation is, however, too short; it would be interesting to know what happened after 1896. And further information is needed as to the prevalence of epidemic disease in the various districts and other local conditions before any conclusions can be drawn from these figures.

It would appear, then, that each of these three statistical methods is beset with fallacies, but it does not follow that a purely statistical method is inapplicable. Where the work of the depot has been conducted on a sufficiently large scale and for a sufficient length of time, conditions which do not appear to have been fulfilled in the case of any existing institution, it may be possible to trace its influence on the mortality figures provided that the investigations are conducted by persons adequately acquainted with the local conditions.

The best method, however, is to institute an investigation—clinical, pathological and statistical—somewhat on the lines of the enquiry lately conducted by Park and Holt in New York¹. The objects of this enquiry were: “(1) To make a comparison of the results of infant feeding in tenements in winter and summer; (2) to determine how far such results were affected by the character of the milk used, especially its original bacterial content, its preparation, and whether it was fed after heating or raw; (3) to see to what extent results were modified by other factors such as the care the infants received and the surroundings in which they lived.” The clinical observations were made during the summers of 1901–02 and the intervening winter by ten physicians, each of whom had a group of about 50 children under observation for periods of about 10 weeks. The children were visited twice a week and their weights taken at regular intervals. No child was ill or suffering from marasmus when the observations began. The total number observed was 632;

¹ Park and Holt, *op. cit.*

340 were six months old or under, 265 from seven to twelve months and 47 a little over twelve months. Many of the infants received breast-feeding at night, and all of course were under regular medical supervision during the period of observation. The statistical results of this enquiry are tabulated below. It should be said that the store milk was in nearly all cases heated in the summer; usually it was raised to nearly boiling point. In the winter in about half the cases some method of partial sterilization was employed. The condensed milk was almost invariably prepared with boiled water.

	Summer observations		Winter observations	
	Good results	Bad results	Good results	Bad results
Store Milk	56 %	44 %	96 %	4 %
Bottled Milk	61	39	94	6
Condensed Milk	60	40	92	8
Milk from Central Distributing Stations	81	19	93	7

These figures are not intended to indicate strict numerical differences. There are obvious statistical objections; for instance, it is not stated whether the age-periods were the same in each group. The observers, however, regard them as comparable, and as indicating a real and marked difference between the results with the milk from the central stations, *i.e.* the Straus depots and similar institutions, and those obtained with the other forms of feeding. The difference is attributed to the fact that at the Milk Depots a certain amount of supervision was exercised over the infants¹, and some systematic attempt at milk modification was made. "Again, what contributed in no small degree to success with this plan of feeding was that this milk was supplied in separate bottles for each feeding, that the quantity for one feeding was suitable for the child, and that only a proper number of feedings for the 24 hours was dispensed at one time. There was not, therefore, the temptation to over-feeding and too frequent feeding which with other methods are so generally practised."

In addition to the statistical reports the physicians were asked to state their own conclusions as to the general problem of infant feeding in tenements. Their opinions founded on their clinical experience are, as Park and Holt say, most suggestive. The unanimous opinion was that the most important factor in securing good results is intelligent care; but as regards the various methods of feeding studied the physicians have no doubt as to the superior advantages of the milk

¹ But all the children in this investigation were under some supervision by the physicians making the observations.

depots. "Of the methods of feeding now in vogue, that by milk from central distributing stations unquestionably possesses the most advantages in that it secures some constant oversight of the child, and since it furnishes the food in such a form that it leaves the mother least to do it gives her the smallest opportunity for going wrong. This method of feeding is one which deserves to be much more extensively employed, and might, in the absence of private philanthropy, wisely be undertaken by municipalities, and continued for the four months from May 15th to September 15th."

This testimony of the New York clinicians is perhaps the best evidence in favour of the Infants' Milk Depot that has yet been published.

Objections.

It may now be useful to briefly consider some objections which have been brought against these institutions. In France they have chiefly been attacked on the ground that they encourage artificial feeding at the expense of breast-feeding. No evidence, however, has been brought forward to substantiate the charge. As a matter of fact, in all the milk depots of which the writer has any knowledge the mothers are distinctly given to understand that the milk should never be used in preference to mother's milk, which they are told is the proper infant food. But the charge of discouraging breast-feeding may be brought with equal justice against all measures which tend to improve artificial infant feeding. From this standpoint the promoters of the *Goutte de Lait* are no more blameworthy than those who seek to improve the condition of the milk supply, who invent appliances to simplify home modification, or educate mothers in the principles of artificial feeding; while the worst offenders are the American physicians, who have done so much to improve and systematize artificial infant feeding. Those who bring this charge against the milk depot must logically apply it to all improvements in artificial feeding, and thus place themselves in the position of the extreme temperance reformers, who regard anything short of the complete extirpation of alcoholic beverages as a mere parleying with vice more productive of harm than good¹. We must

¹ At the same time the writer warmly sympathises with the desire to encourage breast-feeding which animates those who bring this charge. There can be no doubt that the difference between breast-feeding and hand-feeding is very great and implies far more than questions of digestibility and contamination. Ehrlich's theory of immunity has given rise to lines of research the results of which show that profound differences exist between

realize that at the present time artificial feeding is in many cases a necessary evil; the only question is whether it shall be well or badly done—the latter alternative entailing an immense loss of life.

It has also been objected to the English milk depots "that for a municipality to furnish a sterilized milk supply raises special difficulties as to the function of a municipality, which surely should be to control the milk supply and insist upon its purity rather than to itself trade in a sterilized milk¹."

It would seem that objections of this nature would apply equally to a municipal supply of water or to the municipal provision of slaughter-houses, both of which measures are wrong if the function of a municipality be merely to control private traders. And it is questionable whether it is profitable in this connection to consider very closely the difficulties which may arise as to the precise "function of a municipality." In attempting to assess the value of any given administrative measure having for its object the saving of human life it is surely preferable to regard its effect on the public health as the all-important consideration. The question properly falls within the province of preventive medicine, and there appears to be no necessity to enter the region of political science in search of a determination of the "function of a municipality²."

But the controlling and supplying functions are not antagonistic. Liverpool "trades in a sterilized milk," but Liverpool was one of the first municipalities to take action in the reform of the milk supply. The large towns, however, are at a disadvantage, as practically all their milk comes from the country and most of it is already contaminated before reaching the town. In the rural districts the regulations for the control of the milk supply are practically inoperative, and it cannot be the blood and other fluids of different species, and that these differences extend to milk. It is said that mother's milk contains anti-bodies—a fact which explains the immunity of sucklings from infectious disease, and there are probably other fundamental biological differences. It is highly improbable that the milk of another animal can ever adequately replace human milk. It is greatly to be deplored that there has been no organized effort to encourage breast-feeding in this country. Much might be done to imitate the example of the French in this respect. A "Consultation de Nourrissons" should be established in every town as complementary to the Milk Depot. The Factory Act might with advantage be modified so as to extend the period during which a woman is excluded from work after childbirth, provision being made to secure from pecuniary loss all women who could produce satisfactory evidence that they were suckling their infants.

¹ Swithinbank and Newman, *Milk Bacteriology*, p. 503, 1903.

² It will be noted that the New York physicians engaged in the investigation conducted by Park and Holt came to a different conclusion as to the functions of a municipality in relation to the prevention of infantile mortality. See p. 361.

said that there are indications of improvement in the near future. A reliable bacterial standard would remove many difficulties, but the authors of the standard English text-book think that "the time has probably not yet come for fixing a minimum standard of the number of organisms permissible, or for prosecution if that standard be passed¹." Clean milk is, of course, the desideratum, but the difficulties in the way of securing a clean milk supply have been greatly under-rated. It is not merely a question of clean well-constructed cow-sheds. The production of clean milk involves the careful grooming (at 4, or even 2 a.m.) of an excessively dirty animal, and the observance of strict cleanliness of person and utensils by the employees handling the milk. Any observant person who has watched the ordinary process of milking, and noted the standard of cleanliness of cows and milkers, must surely agree that if manure and other filth is to be kept out of milk it will be necessary either to effect something like a revolution in the personal habits of the employees in the milk trade, or to substitute persons of a higher social standing, who may be relied upon to maintain the necessary standard of cleanliness and observe a reasonable reticence in the matter of expectoration in the absence of external compulsion.

But assuming these difficulties in the way of clean milk production by private enterprise to be solved, at what price will the milk be sold? Chapin, who writes with authority on this subject, appears to regard a rise in price as inevitable and the attempt to improve "grocery milk," therefore, as "almost hopeless."

"Legislation cannot compel a farmer to produce his milk at a loss, and the population that consumes 'grocery milk' would vote out of office authorities that prohibited its sale or advanced its cost"..... "Farmers cannot be expected to take additional care of their milk without extra compensation. Higher prices to the farmer is the solution of the milk problem, and the dealer should also have extra compensation for any additional labour and care on his part²."

The fear of being "voted out of office" exists on this side of the Atlantic, and is one of the factors to be reckoned with by the reformer. If the agitation for "clean milk" results in raising the price of milk so as to take it beyond the reach of the poor the milk reformers will have done more harm than good.

Let it be granted, however, that clean milk at ordinary prices is brought to the door of the consumer, is the problem of the prevention

¹ Swithinbank and Newman, *op. cit.*

² Chapin, *The Theory and Practice of Infant Feeding*, 1903.

of contamination solved? By no means, for, as we have already seen, in diarrhoea the contamination takes place largely, if not chiefly, in the home of the consumer. One of the chief advantages of the milk depot is that the milk is supplied in such a way as to reduce the possibility of contamination within the home to a minimum. But here another objection is raised. It is contended that home contamination is best prevented by educating mothers and the elder school-girls in the methods of artificial feeding, and in the care of infants generally¹. Provided that the curriculum includes instruction in the enormous advantages of breast-feeding—a point somewhat neglected by English writers—the writer fully accepts this statement, but he would point out that there is no antagonism between the Milk Depot and education. On the contrary the depot is in itself an important educational influence. It is, as Variot urges, “Une école d’Alimentation.” And the diseases due to ignorance are far from being limited to those depending on imperfect infant feeding. The prevalence of tuberculosis and small-pox would be immensely diminished if the public were adequately instructed as to the proper preventive measures, but pending the arrival of that period of enlightenment a compulsory vaccination law has its uses even in Germany. At the present time it is unsafe to rely on educational methods only for the prevention of any disease.

We may conclude, then, that the real objects in connection with artificial infant feeding at which the sanitary authority should aim are (1) clean milk, and (2) the education of present and future mothers, but that the attainment of these objects is a matter of very great difficulty; and if it be admitted that pending such attainment the Infants' Milk Depot fulfils a useful function we must also conclude that its period of usefulness will certainly be protracted.

Another objection brought against the English depots is that inadequate provision is made for the medical supervision of the infants. It is urged that regular weighing and inspection by a medical practitioner should be insisted on in all cases, as in some of the *Gouttes de Lait*. Niven has some valuable suggestions on this point². It must

¹ Swithinbank and Newman, *op. cit.* See also Niven, *Annual Report on the Health of Manchester*, 1902.

² *Op. cit.* Niven's suggestions as to the methods on which a milk depot should be conducted are as follows:

1. The milk must be obtained from farms kept under inspection by the Corporation, and preferably managed by the Corporation.

2. When modified and sterilized it should be sold at paying prices except to persons willing to submit to certain rules.

be confessed that improvements in the practice of the depots in this respect are desirable, but at the same time it is important to avoid anything like undue restriction, otherwise a prejudice against the depot is raised. It does not follow that methods which are practicable in France will succeed in this country, where there is a strong feeling against official regimentation. And it is important that any steps in this direction should be taken with the cordial co-operation of the local medical practitioners; otherwise success will certainly not attend the depot.

Another objection deserves consideration. It is contended that when a municipality undertakes a supply of milk, the sources of the supply should be subject to strict supervision and control, and that only milk of a high degree of bacteriological purity should be used. This is, of course, indisputable, and it must be admitted that there is room for improvement in this respect. In the Straus depots in New York, however, the milk is certified by the Milk Commission and is of a high standard of purity. In some of the Gouttes de Lait great care is exercised to obtain clean milk. The milk for the Rouen Goutte de Lait comes from a model cow-shed where the conditions are excellent, and is transported to the city in special motor-waggon¹. In Liverpool and Battersea, to name only two of the British depots, the contractors are under special regulations.

But it must be admitted that it is easier to lay down regulations than to ensure their fulfilment, and the writer entirely agrees with Niven's suggestion that the farms from which the milk for a municipal milk depot is drawn should be municipal institutions under the direct

3. These rules should include systematic weighing of the infant, inspection of the house, and supervision by officers of the Corporation.

4. A written undertaking must be given to keep up the feeding of the child for a period of not less than three months with milk obtained from the depot; to give the infant no other food whatever, and if it is desired to give up the method of feeding to lodge at the Health Office a statement of the reasons why the mother desires to be released from her undertaking.

5. The person assisted must also undertake to carry out the other instructions given by the visiting officer of the Corporation in the management of the infant.

6. The Corporation to provide adequate means of supervising the application of the milk supplied, and to keep a record of the condition of the children supplied with modified and sterilized milk.

7. Where the milk is supplied at a paying price no supervision should be maintained or agreement entered into.

¹ Une Vacherie modèle annexée à la Goutte de Lait de Rouen, *La Clinique Infantile*, Paris, January 15th, 1904.

management of the responsible officers of the sanitary authority. In this connection it is interesting to note the experience of the city of Rochester, N.Y., U.S.A. In 1897 the municipality began a supply of milk on methods similar to those afterwards adopted in this country. At first the milk was sterilized or pasteurized, but in 1899 the following plan was adopted:

"A central station at which the milk is prepared is organised each season on a farm outside the city, where a trained nurse and assistants have full control of the cows, utensils, bottles, etc., and where all of the milk work is carried on in a portable milk laboratory. Everything coming in contact with the milk is thoroughly sterilised in steam sterilisers. The milk itself is not subjected to any pasteurising or sterilising process. Sterilising and pasteurising are only an open invitation to the milkman to be careless in the production and handling of milk.

"At the milk station on the farm the milk is taken from clean, well-fed, tested cattle into sterile cans, which are carried to the barn in sterile cheesecloth bags. Just before milking the cows' udders are washed. A sterile cheesecloth fly cover is placed over the cow, the first portion of the milk being rejected. So soon as the cans are filled they are immediately covered by a layer of cheesecloth held in position by a rubber band. The cans of milk thus covered are immediately taken from the barn into the laboratory, about 200 yards away, where the milk is properly diluted, sweetened, and turned off into sterile nursing bottles of various sizes of the Siebert type. The bottles are corked with sterile rubber corks, placed in racks, covered with cracked ice, and immediately transferred to the city for use. Of the cleanliness of milk prepared in this way, 43 daily samples were found to average not more than 14,000 bacteria per cubic centimetre, while city milk for the same period approximated 235,000 bacteria per cubic centimetre¹."

These methods are far in advance of anything which has been done by the British municipalities, and it is much to be hoped that they will be introduced into this country. Not only would the necessity of any heating process be removed (and with it the charge of disseminating scurvy², a danger which has been greatly exaggerated) but an object-lesson could be given in methods of clean milk production which would give an immense impetus to the movement for the reform of the milk supply now on foot in this country.

¹ "The Influence of the Municipal Milk Supply on the Deaths of Young Children." Dr Goler, Health Officer, Rochester, N.Y., U.S.A., *New York State Journal of Medicine*, December 1903.

² Ashby, *British Medical Journal*, February 27th, 1904.

APPENDIX.

Dr Dufour gives the following list of towns in which Gouttes de Lait are either projected or actually established¹:

France (65 towns) as follows:

Aix—Albert (Somme)—Alger—Alençon—Amiens—Alfortville—Anzeville par Cartenay—Avignon—Barentin—Bellevue (Seine)—Besançon—Bolbec—Bordeaux—Bourgoin (Isère)—Biarritz—Bléville (près Le Havre)—Beauvais (Oise)—Boulogne-sur-Mer—Bourg—Caen—Cahors—Castres—Cambrai—Chateaufort (Nièvre)—Châlons—Châtellerauld—Clermont-Ferrand—Deville-les-Rouen—Dunkerque—Elbeuf—Epernay—Jouy-en-Josas—Le Havre—Le Mans—Lille—Les Ponts-de-Cé (Maine-et-Loire)—Lobrosse (près Auxerre)—Lorient—Melun—Marseille—Moulin—Nantes—Nancy—Nice—Orléans—Paris—Poitiers—Rouen—Reims—Rennes—Roanne—Saumur—Sarlat—Saint-Just—Saint-Nazaire—Saint-Martin-Vésuby—Saint-Pol-sur-Mer—Saint-Germain-en-Laye—Tours—Toulon—Toulouse—Tourcoing—Versailles—Valence—Vinsobres (Drôme).

Other European countries (35 towns) as follows:

Antwerp—Athens—Battersea—Berlin—Bucarest—Bigheur (Belgium)—Brussels—Barcelona—Cadiz—Crasiova (Roumania)—Corélare (Portugal)—Düsseldorf—Florence—Gand—Geneva—Halle—Königsberg—Lisbon—Liverpool—Lozen—La Haye—Mannheim—Madrid—Naples—Odessa—Pforzheim (Baden)—Parbou—Rome—Stockholm—Schwabgmund—Saint Helens—Tenneswey—Turin—Varna (Bulgaria)—Zurich.

America: Buenos Ayres—Santiago—Montevideo—Montreal.

Asia: Bombay.

Africa: Canary—Constantine.

In addition to the above list, Milk Depots have been established in the following towns:

Great Britain: Ashton-under-Lyne—Bradford—Dukinfield—Leith—York. The Corporations of Glasgow and Dundee have decided to establish Milk Depots.

United States: Chicago—New York—Philadelphia—Rochester, N.Y.—Yonkers, N.Y.

¹ *La Clinique Infantile*, Paris, November 1st, 1903.

EXPLANATION OF PLATES XVIII.—XXIV.

PLATE XVIII. This illustration indicates the three main features of the work of the Goutte de Lait, viz. (1) systematic weighing of the infant, (2) regular medical supervision, (3) distribution of sterilized milk. This Goutte de Lait was established by Dr Variot in 1892 at the Belleville Dispensary, Paris.

PLATE XIX. The Brussels Goutte de Lait or "Laiterie Maternelle" was founded in 1897 by Dr Eugène Lust. This illustration is reproduced from Dr Lust's *Contribution à la Puériculture*, Brussels, 1903.

PLATE XX. The Liverpool Corporation's Depot at Earle Road was opened in 1902. It is the largest and most completely equipped depot in this country.

PLATE XXI. In this illustration the machine for weighing the babies is shown on the lowest shelf. Affixed to the second shelf is the butyrometer by means of which the milk is tested for fat every morning.

PLATE XXII. The task of filling several thousands of small bottles daily involves considerable labour even when a good machine is used. In this appliance the milk is first poured into the large receiver at the upper part of the machine whence it flows into the row of cylinders. When the handle at the right of the machine is pulled the milk is discharged into the bottles below. The quantity per bottle for each set of bottles can be easily regulated.

PLATE XXIII. In this plate the six different quantities per bottle supplied at the Liverpool depots are indicated. The stopper of each bottle is protected by an adhesive printed label before leaving the depot.

PLATE XXIV. This illustration shows the loaded trolley entering the sterilizing chamber. The door is then drawn down and securely clamped, and steam is injected through the pipe in front of the machine. The bottle-filling appliance is shown standing on the left-hand trolley.

PLATES XX.—XXIII. are from photographs kindly supplied by Dr Hope, Medical Officer of Health of Liverpool; Plates XX. and XXI. appeared in Dr Hope's Annual Report on the Health of the City of Liverpool, 1901. The writer's thanks are due to Dr Hope for permission to reproduce these plates, and to Dr Lust for permission to reproduce Plate XIX.



"L'Œuvre de la Goutte de Lait" (Belleville Dispensary, Paris).

From the painting by M. Jean Geoffroy, exhibited in the Paris Salon in 1903, now the property of the municipality of Paris.





Group of mothers and babies in the "section gratuite" of the "Laiterie Maternelle," Brussels.





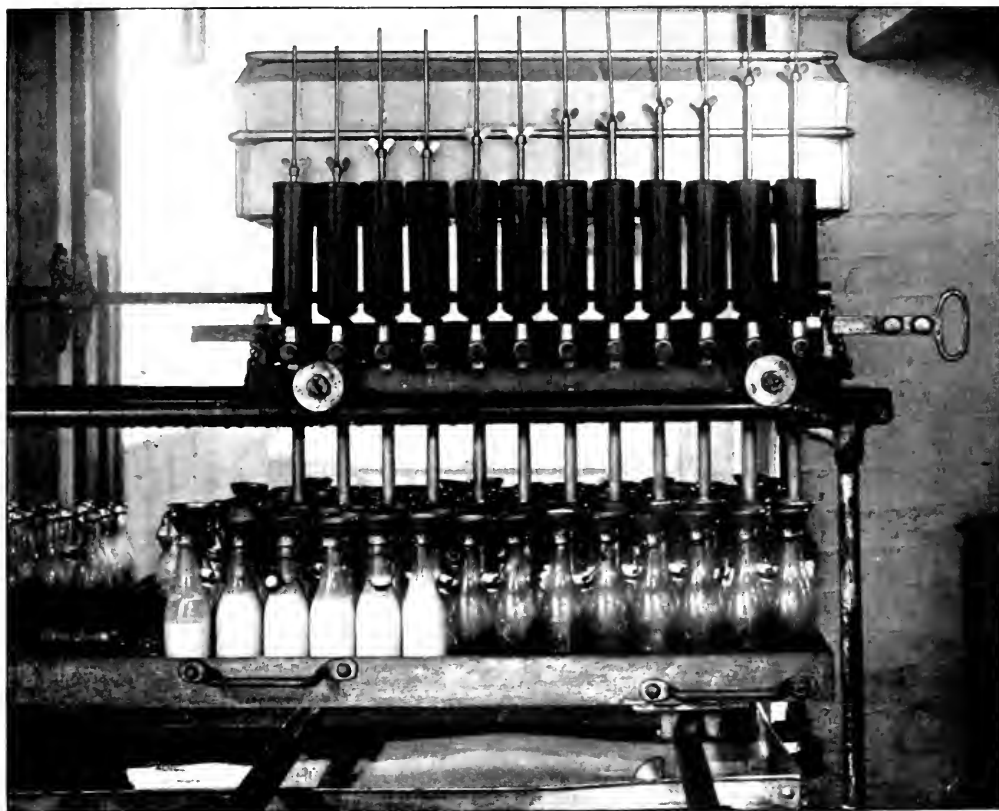
Exterior of Infants' Milk Depot, 52, Earle Road, Liverpool.





Infants' Milk Depot, Earle Road, Liverpool. Corner of interior of Shop.





Infants' Milk Depot, Earle Road, Liverpool. Bottle-filling machine.



Bottles of milk supplied at the Liverpool Milk Depots, showing the different quantities supplied to children at different ages.





Infants' Milk Depot, Battersea. Interior of sterilising room, showing steriliser with loaded trolley.



HAY FEVER; RECENT INVESTIGATIONS ON ITS CAUSE, PREVENTION, AND TREATMENT.

BY R. ASHLEIGH GLEGG, M.B., CH.B., D.P.H. EDIN.

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PART I.*Introduction.*

HAY FEVER is a disease which on account of its peculiar interest has attracted the attention of the medical world ever since it was first accurately described by Bostock of London in 1819. To what extent the disease occurred before his time is difficult to determine; but from the fact that his observations were at once confirmed by a number of contemporary medical men it is probable that at the time of his writing it was not very uncommon. We have no exact knowledge of the existence of the disease in England prior to the nineteenth century, and indeed many authors hold that the chief etiological factors were not present in earlier times. It is my purpose in this paper to discuss the researches recently carried out by Prof. Dunbar at the State Institute of Public Health in Hamburg, which have very considerably increased our knowledge of the interesting malady, and have also narrowed the field for its future investigation.

Hay fever occurs yearly in individuals, with an inherited or acquired predisposition, only at a definite period of the year, and not outside of that period. In England, Germany and other countries of middle Europe it appears about the middle of May, and lasts until about the end of July; whereas in the United States of North America the disease is seen at different periods, *e.g.* in the Northern States it occurs typically in the early summer, and again in the autumn, the earlier form being called 'June cold' or 'Spring catarrh,' and the later is known as 'Autumnal catarrh,' which beginning in August or September lasts until the first frost. In the Southern States and notably in New Orleans cases are met with all the year round. The reason for these differences will be apparent when the etiology has been considered. Whenever the season is passed the symptoms disappear, and the patients may count on ten or eleven months of perfect immunity from the disorder. While the hay-fever season reigns, on the other hand, the sufferers pass a very miserable time, being quite incapable of attending to business affairs, and unable to enter into any of their usual amusements, since the slightest exposure in the summer air may bring on the symptoms of the disease. The illness is indeed of no trivial nature, especially when it occurs as a complication of graver diseases, and means to combat it successfully will be a god-send to the

many thousands of patients who otherwise look forward with dread to the most charming season of the year.

The symptoms of hay fever, which are apparently very similar wherever the disease occurs, are somewhat as follows:—A few weeks before the hay-fever season approaches, the patients notice at times a slight feeling of itching in the eyes and nose, and transient slight catarrh of the upper air passages. Then one day there comes on suddenly, either a sneezing fit, or else a strong feeling of itching and burning in the eyes and nose, followed by most profuse lachrymation and rhinorrhoea. Fluid positively streams from both eyes and nose, and it is no exaggeration to say that in certain cases four or five handkerchiefs in as many minutes are one after the other soaked with secretion. The sneezing fits are of most remarkable intensity, not one or two isolated sneezes, but paroxysms of the most painful kind, which the patient is quite unable to control. The inclination to rub the itching parts is irresistible, and there soon follows marked injection of the conjunctival vessels, with all the symptoms of acute conjunctivitis. The eyes become hot and red: this is followed frequently by chemosis, and the lids become so swollen that the patient can scarcely see. Later there appears swelling and congestion of the nasal mucous membrane, so great as to completely prevent the passage of air through the nose. Longer exposure in the hot dusty summer air leads to a spread of the process. There is itching of the gums, which makes many patients grind their teeth most unpleasantly, also intense itching of the fauces with short dry cough. A few notice a sharp pain in the ears. As the process increases in intensity, it spreads in some patients to the deeper air passages, causing a feeling of oppression in the chest, and symptoms more or less of an asthmatical character. This asthma, in severe cases, has been known to produce emphysema, and constitutes by far the most troublesome and dangerous symptom. Besides these symptoms every hay fever patient has during the attacks a very great feeling of lassitude and particular irritability of temper, and suffers also frequently from headache and sleeplessness. Of less common phenomena may be mentioned urticaria-like skin rashes attended with great itching. Fever as a rule is not present, although it does occur to a certain extent in some cases. The severity of the attacks depends to a great extent on the weather. In rainy weather the troublesome symptoms quickly disappear, and do not return so long as the surface of the ground remains damp. By staying in rooms with the doors and windows kept shut the attacks are less severe and less frequent. In localities with scanty vegetation

and at the sea-coast hay fever only very exceptionally occurs. It appears in its worst form after enforced exposure in the streets and dusty roads in hot, sunny weather, and very particularly on railway journeys after sitting by an open window.

The symptoms of hay fever are more apt to call forth the ridicule than the sympathy of onlookers, and it is a well-known fact that patients are too often made the laughing-stock of their friends. Yet it must not be forgotten that this malady becomes of grave moment when it occurs as a complication of more serious diseases. The nervous symptoms, for instance, may, and often do greatly aggravate cases of neurasthenia. The sneezing and cough, and even more the asthma and emphysema, necessarily become a great danger in cases of heart and lung disease. Physicians realise only too well the grave significance of hay fever in elderly patients with arterio-sclerosis. Cases of phthisis are known to be most injuriously affected by the frequent coughing and by the stress of the sneezing fits. In one case of latent phthisis a recrudescence of the disease was observed whilst the patient was suffering from hay-fever attacks. We shall refer later in detail to this case.

Some Factors in the Etiology.

Geographical Distribution. As regards the distribution of the disease, accurate statistics of the relative number of cases in the different countries are difficult to obtain, partly owing to faulty diagnosis, and partly to the desire of many patients to conceal the fact that they suffer from it, fearing to be considered neurasthenic. It was formerly said that the disease chiefly affected the Anglo-Saxon race, and indeed the majority of cases appear still to occur in England and America. It is rare in the north of Europe, and the number of patients in France, Russia, Italy, and Spain appears not to be very large. In Holland there are more, and in Germany, judging from the correspondence to be later referred to, and from the fact that there has been founded a Hay Fever Association of Heligoland with already about three hundred members, the number of cases would seem to be considerable. Indeed it has been said that at the present day there are in every large town in Germany almost as many hay-fever patients as in the Heligoland Association. Cases are not known to occur in Asia or Africa outside the European population.

Heredity. The influence of heredity is marked in the disease. In Beard's ⁽⁶⁾ experience it was a factor in 33 % of his cases. Instances of

the transmissibility of the hay-fever predisposition have been recently observed, where the disease has been acquired by an adult belonging to a family not previously affected by it, and where the patient's children already show unmistakeable signs of the disease.

Sex. It is the general opinion of authors that as a rule more men than women suffer from hay fever, the proportion being about 2:1; but statistics on the matter are not at all satisfactory.

Age. The disease has been observed to appear as early as the second year, and as late as the sixtieth; but most of the cases occur between the years of ten and forty. The disease belongs rather to the early and middle periods of life, but it is noticed that as a rule the attacks gradually increase in severity as age advances. A certain number of patients however apparently have the opposite experience.

At any rate in the majority of cases it does not appear materially to influence the prospect of a patient's longevity.

Constitution. It has already been said that hay fever occurs in persons with an inherited or acquired predisposition, and this predisposition is one of the most remarkable and interesting features of the disease; yet although much information on all the circumstances of a very large number of hay-fever patients has already been collected by different authors, in particular by Phoebus of Giessen⁽²⁶⁾, and Beard⁽⁶⁾ of New York, definite conclusions as regards the nature of the predisposition have not been arrived at. It is only therefore possible to pass in review certain facts and opinions having a bearing upon the subject.

Temperament. Most authors hold that hay fever occurs in those of active energetic type, yet a few maintain with Wyman⁽²⁵⁾ that it attacks persons of all temperaments.

Education and Social Position. A high degree of mental development has evidently a marked influence in predisposing to the disease, since the great majority of cases occur amongst the educated, and in people of good social position. In opposition to this view some hold that it passes unnoticed amongst the poor, who are not given to accurate observation. Those, however, who have seen the disease, and still more those who suffer from it, hold that the symptoms of real hay fever can scarcely escape observation.

Suggestion. We find others, who did not differentiate the disease sufficiently from nervous coryza, believing that hay fever is caused, and can be brought on by pure suggestion. Of recent observers Thost⁽²⁴⁾ of Hamburg made a careful enquiry into the complaint, noticing the

frequency of its occurrence amongst men of learning, especially after severe bodily and mental exertion, also its establishment in cases of exhaustion following many of the infectious diseases, particularly after influenza, and its occurrence after difficult labours.

A number of authors, *e.g.* Gueneau de Mussy and Molinié⁽²¹⁾, regarded the special predisposition to hay fever as the outcome of a gouty or arthritic diathesis. These diatheses are indeed present in many instances, and in England especially gout and hay fever are frequently found in association amongst the rich and better educated; but hay fever cannot be explained as the result of the gouty diathesis, for the disease occurs in very many who have no gouty tendency. Thost⁽²⁴⁾, for instance, in an examination of 400 cases found a gouty disposition only in thirty.

Former Theories of the Etiology.

Hay fever affords yet another illustration of the familiar fact that the number of theories concerning the etiology of a disease is in inverse proportion to the state of knowledge of the subject.

That the malady was the outcome of local abnormalities, or diseased conditions of the nose was the opinion of Daly, Allen⁽³⁾, Roe, and others. Many, amongst whom were Sajous, Hack⁽¹¹⁾, J. N. Mackenzie⁽¹⁹⁾, and Fink⁽¹⁴⁾, held that a diseased condition of the endings of the fifth nerve was responsible for the symptoms of hay fever.

These pathological theories appear, at first sight, as quite possible explanations of the phenomena of the disease. The first of these, *viz.* that hay fever depends on gross structural changes within the nose, was critically discussed by Morell Mackenzie⁽²⁰⁾. He showed that the theory is open to the following fundamental objections; that in the first place only an insignificant proportion of the many sufferers from nasal disease are hay fever patients; that in many of the worst cases of hay fever there is absolutely no evidence, objective or subjective, of disease within the nose, and lastly that this doctrine altogether fails to account for the limitation of the complaint to one, or at most two brief seasons of the year. The theory that the trigeminus is responsible for the disease will be discussed at a later stage.

Most of the theories summarised above were formulated to explain the manifest predisposition that the hay-fever patient has to his complaint. As to the exciting cause which regularly every summer brings on the illness, there was a like diversity of opinion amongst

former observers. A micro-organismal theory was proposed by Helmholtz. Foremost amongst his many followers was Sticker⁽²⁷⁾, and of late Heymann and Matchusita⁽¹³⁾ have made observations on the same lines. It was natural from a study of the symptoms and character of the disease that the theory of mechanical irritation of a specially sensitive mucous membrane should have been widely entertained. Bostock⁽⁷⁾, Phoebus of Giessen⁽²⁶⁾, and Beard⁽⁶⁾, amongst others, variously ascribed it to dust, bright light, heat, odoriferous substances, etc. The popular view was that the symptoms of hay fever are produced by emanations from hay, or by the pollen of grasses. Experiments with pollen, with the view of determining the value of this popular opinion, were carried out by two former observers. These were Elliotson⁽²⁸⁾, who published his work in 1831, and Blackley⁽⁴⁾, whose researches were conducted about forty years later.

Elliotson believed that the disease does not usually appear "till the grass comes into flower, and as long as there is any flower remaining on the grass the disease continues."

Blackley examined the pollen of various grasses, and of plants belonging to thirty-five other natural orders. He devised a most ingenious method for counting the number of pollen grains in the air and found that in England 95% of the pollen contained in the atmosphere belongs to the Graminaceae. His experiments were very carefully conducted. Pollen was applied to the mucous membranes of hay-fever patients, and was found to cause the same symptoms as in the natural disease, both in its catarrhal and in its asthmatical form. He concluded that hay fever is produced by the pollen of the grasses chiefly, but that a like power, with rare exceptions, was in some degree also possessed by the pollen of all the plants he examined. He believed the disturbance caused by pollen to be due partly to its mechanical, and partly to its physiological action,—the pollen tubes penetrating the mucous follicles, and thus giving rise to irritation. He further thought that volatile oil might be a constituent of the pollen, and might commence the disturbance. Finally he speaks of the valuable information which would probably be derived from a study of the chemical constitution of pollen grains, to determine whether there be not present "some powerful substance of the nature of one of the poisonous alkaloids, or some other equally powerful class of bodies."

A portion of the results obtained by Blackley were corroborated by experiments made by Morell Mackenzie⁽²⁹⁾ in 1887. These researches of Blackley, in particular, were thus very suggestive so far as they went,

and made a very valuable contribution to the knowledge of the subject: yet during a period of thirty years, after his work was published, although much had been written about the etiology of hay fever, and many new theories had been formulated, no really definite etiological factor explaining the peculiar phenomena of the disease in all cases was arrived at.

Now in approaching the study of the results of the most recent researches on the subject, we must agree with Dunbar's⁽⁸⁾ proposition that correct conclusions in regard to the cause of hay fever will only be arrived at when it is possible to separate the factor completely from foreign elements, and by means of this factor, independent of temperature and meteorological conditions, and at a time of the year different from the real one, to cause all the symptoms of the disease, and that only in persons predisposed thereto.

The Results of Recent Researches.

Dunbar instituted experiments with the pollen of grasses, and other plants, in the spring of 1902. The results of these will now be reviewed.

Pollen of rye, carefully collected, free from bacteria, and applied to the eyes and nostrils of a large number of hay-fever patients, called forth *typical hay-fever symptoms*, both in the eyes and nose. These symptoms included photophobia, and a feeling as of sand in the eye, lachrymation, injection of the conjunctival vessels, oedema of the lids and even chemosis. The nasal mucous membrane became greatly swollen, along with much itching and rhinorrhoea. A large number of normal individuals were experimented on without any such effect.

That this action was produced by a specific poison in the pollen, and not by any hypersensitiveness of the trigeminus, according to the theory of J. Mackenzie and others above referred to, was proved by the fact that the same active pollen applied to the anal mucous membrane of a hay-fever patient, produced in that situation severe itching which lasted some hours. A similar experiment with a person not subject to hay fever was without result.

This specific irritation of the mucous membranes of hay fever patients was not excited by the mere mechanical irritation of the pollen, for rye pollen, which is particularly toxic, has a perfectly smooth outer surface, and other pollen examined, which had a prickly surface, did not give rise to the above reaction. Mechanical irritants of various

kinds, such as soot, dust, etc., were also experimented with, but characteristic symptoms were not produced in patients predisposed to hay fever by such means.

Many experiments were made to exclude the possibility of suggestion. At all times of the year, even in mid-winter, it was found that the disease could be brought on artificially in hay-fever subjects, through the agency of the pollen of certain plants.

These results, showing the peculiar specific poisonous nature of some varieties of pollen, are all the more interesting, as Dunbar⁽¹⁰⁾ in commencing his investigations with the view of clearing up the obscurity of the etiology of hay fever, inclined to support the theory that micro-organisms set up the attacks; and indeed as the result of many examinations he found the continuous, exclusive, and abundant presence of certain bacteria, in the nasal and other mucous secretions of hay fever patients. Further observations however led him to disbelieve in the etiological significance of bacteria in the disease.

He suffers himself from hay fever, and noticed that attacks excited during the hay fever season promptly subsided when he withdrew himself into a room where all the doors and windows were shut, only to come on again after exposure in the open.

Observations of this nature were carried out on railway journeys during the hay fever season; attacks coming on after sitting by an open window, especially when the train was passing through the midst of cornfields in warm weather, and these attacks passing away when windows and ventilators were closed.

This disposed of the possibility of a micro-organismal infection, where growth and multiplication of the germs would certainly have led to an increase and not to a decrease of the symptoms when the entrance of outside air was discontinued.

Experiments with pollen were then instituted, with the highly interesting results already described. A further experiment of much interest was that in which two persons, one a hay-fever patient, the other not subject to the disease, were placed in a glass chamber. Each blew into a flat dish on which a small quantity of rye pollen had been placed. The hay-fever patient, after a deep inspiration, caused by the act of laughing, was attacked by cough, accompanied by dyspnoea with inspiratory stridor, and suffered for two days from bronchitis, with purulent sputum. The other person showed no symptoms of any kind.

Up to the present time the pollen of 130 different plants has been examined with regard to effects on persons liable to hay fever. The list

of 114 plants tested by Dr Kammann at the Hygienic Institute, Hamburg, which includes all those with toxic pollen, will be found grouped in the Appendix. He adopted the Linnean classification, and examined plants from most of the classes and most of the orders of that system. Plants with potent toxic pollen chiefly belong to the third, nineteenth, and twenty-first classes of this system. Of these pollens those of 25 grasses and only 7 of other kinds of plants (see Appendix) excite a positive action. In these other plants the pollen is not so potent as is that of the grasses. The pollen of rye was found to be most active. The pollens of all the other plants tested were without influence of any kind. They appeared to be quite inert. Experiments on the toxicity of different pollens are still being carried out.

Liefmann, in a research conducted in the Hygienic Institute, Hamburg, the complete results of which will shortly be published, confirmed Blackley's statement, that on days when attacks of hay fever are especially severe there is an unusually large amount of pollen in the air; that, in fact, the severity of hay-fever attacks is in direct proportion to the quantity of pollen present in the atmosphere. He further proved that the amount of pollen inhaled by a patient on days when hay-fever symptoms were present was more than sufficient to induce attacks; for it has been demonstrated that the quantity of toxin yielded by two or three pollen granules suffices to cause a distinct hay-fever attack in some predisposed subjects. Liefmann found indeed that on certain days a patient would inhale on an average from each cubic metre of air about 700 pollen grains.

The Structure and Chemical Constitution of Pollen.

A grain of pollen, examined microscopically, exhibits the characters of a simple cell, of definite and regular shape, averaging about 50—60 μ m. in length, whose contents are enclosed by a double membrane.

The outer coat, or exine, is pierced by one or more holes or slits, the pores, and is either smooth or more or less densely covered by minute and very fine protuberances. As has already been said, pollen, which has been examined and found active in inducing attacks like hay fever, has a smooth exterior; whilst the pollen whose outer membrane is rough, has been found to be inert. *Solidago* pollen is an exception, it having a rough surface.

The outer membrane of rye pollen is smooth. When treated with

Millon's reagent however it shows a reticular structure. It stains with aniline dyes. The inner coat, or intine, which remains unstained by iodine and aniline dyes, becomes distinct when treated with a solution of chromic acid. It is probably a cellulose membrane.

The contents of the pollen cells, which were found to be toxic, consist of granular matter that stains a dark blue with iodine solution and gives other reactions showing it to be of an amyloid character. This granular matter magnified five hundred times presents a very striking resemblance to bacteria, having the same form, size, and staining reaction; and such bodies found in nasal mucus of a hay-fever patient might very readily be mistaken for bacilli.

20 million rye pollen grains weigh about one gramme.

The chemical constitution has been thus estimated by Kammann, in air-dried rye pollen :

Water 10·18 %_o. Organic Matter 86·4 %_o. Ash 3·4 %_o.

The organic matter consists of fats, albuminous substances, nitrogen-containing substances not of an albuminous nature, carbohydrates, and enzymes. These latter are proteolytic and diastatic. The question as to whether the toxic reaction resulted from these enzymes was settled negatively by Dunbar as follows :—He had taken about five grammes of maize pollen with him on a journey. On the way this pollen became fluid, owing it was thought to bacterial decomposition. On this supposition a small quantity of carbolic acid was added to check bacterial growth. Experiments with this material at a later date showed that the pollen was quite inert, but that there were still active enzymes present. It was not found possible to destroy the ferments without also rendering the toxin inert. Attempts to distinguish by chemical means the toxins obtained from all the different plants with toxic pollen will probably not be possible.

Experiments at first seemed to show that the toxin was destroyed when exposed to a temperature of 70° C. These experiments were carried out by Dunbar at a time when only a very small quantity of pollen was available, and later investigations with larger quantities of pollen gave more accurate results. These results, showing the effect of different degrees of temperature on the toxin, may be tabulated as follows :

1.	Temperature	60—70° C.	No diminution in toxicity.
2.	„	70—80°	Toxicity now $\frac{3}{4}$ of original.
3.	„	80—90°	„ „ $\frac{3}{4}$ „ „
4.	„	90—100°	„ „ $\frac{1}{4}$ „ „

The effect of acids and alkalies on pollen toxin has also been ascertained. After being treated with sulphuric acid the proteid remained still very toxic. Alkalies had much greater effect in diminishing toxicity; they rendered the toxin nearly inert.

Isolation of the Toxin.

Following on the results showing the special toxic action of pollen an endeavour was made to isolate the poisonous constituent.

After destruction of the limiting membrane of the pollen grain, it was found that pollen becomes in great part dissolved in nasal and lachrymal secretions, in serum, and in physiological salt solution, kept at body temperature.

Pollen applied to the dried skin had no irritant action, but when the skin was moist with sweat then the pollen set up irritation with itching in subjects predisposed to hay fever.

It is unnecessary to enter into the details of the original experiments, but in brief the perfected process by which the toxin is obtained is as follows:—Pollen is extracted in saline solution at 37° C., for some six to twelve hours, and then precipitated with alcohol.

In the proteid obtained Kammann found that the globulin is inert, whereas the albumin is highly toxic. So toxic is it, that so small a quantity as a forty-thousandth of a milligramme of the common proteid, in solution, locally applied in the conjunctival sac of a hay-fever patient, is capable of causing itching and redness lasting for some hours. It was furthermore proved that the other constituents of the pollen grains, including the ethereal oils, do not possess this special action.

Experiments with the Toxin.

Toxin locally applied on a very large number of hay-fever patients and control subjects, produced, in the predisposed, characteristic hay-fever symptoms, subjective and objective, varying in amount, both in the eyes and in the nose.

The special action of the toxin is a yet further proof that the reaction is due to chemical, and not to mechanical agency. This is rendered still more obvious by the results of subcutaneous injection of toxin, which produced in a patient all the symptoms of hay fever, in typical and severe form. There were induced lachrymation, sneezing, cough, roughness of the throat, inspiratory stridor, and blocking of the

air passages, besides other even graver symptoms. Subcutaneous injection of the toxin in a control subject on the other hand produced no such effect.

Dunbar has thus brought forward convincing evidence that the exciting cause of hay fever in all the cases coming under his observation is a toxin contained in the pollen of certain plants, particularly in that of rye and other grasses.

Attention was then turned to the disease as it occurs in the United States of North America. There, as has been already said, hay fever appears in the different States at different seasons of the year. In the Northern States, for example, two forms are observed, one occurring in the early summer, and the other in the autumn. Dr Dunbar had the opportunity himself of experimenting with four American patients, who suffered while in America from the latter form (autumnal catarrh). These patients did not suffer from the attacks of the natural disease in Germany, at the time of its prevalence there. He found that they were not at all, or only slightly affected by the toxin obtained from rye, but were at once seized with severe and typical appearances of the disease when the pollen or the toxin from *Ambrosia* (Wormwood) and *Solidago* (Golden Rod), late-flowering American plants of rank growth, was applied in the same manner as in the other experiments to the mucous membranes of the eyes and nose.

Preparation of Pollen Antitoxin.

After isolating a toxin of such special nature Dunbar naturally endeavoured to obtain an effective antitoxin. This antitoxin was first obtained by injecting the pollen, later the toxin derived from the pollen of various grasses and other plants, into different animals, such as rabbits, goats, and horses. The animals reacted very differently to the poison, and young thoroughbred horses were as a rule found to be best suited for the purpose. The antitoxin obtained was capable of neutralising the toxin *in vitro*, so that a mixture of toxin and antitoxin applied to the conjunctivae of persons previously reacting to the toxin, now gave rise to no irritation. It was also found possible by means of the antitoxin to allay artificial attacks excited by toxin and pollen.

A question of great importance, on which the practical possibility of treating the disease successfully by means of the antitoxin depends, is whether the toxins of the various pollens proved active are alike? Although not identical it is certain that they are very closely related

to one another, for it is found that antitoxin obtained from the pollen of one sort can neutralise, though not always in the same degree, toxins derived from other pollen. The toxic proteid for instance from *Solidago* pollen was neutralised *in vitro* by maize pollen antitoxin. This was also demonstrated in cases of American autumnal catarrh, where the artificial attacks induced by the *Solidago* pollen were allayed by means of pollen antitoxin obtained from the Gramineae. It is of further interest that irritant symptoms of varying intensity were also produced in six hay-fever patients in Germany, when the pollen or toxin of *Solidago* and *Ambrosia* was applied to their conjunctivae, and that these appearances were quickly removed by means of maize or rye pollen antitoxin.

General evidence that Pollen Toxin is the exciting cause of Hay Fever.

In seeking a confirmation of these important results specimens of pollen and of toxin and antitoxin were sent to medical men, not only in Germany, but also in England and Scotland, Denmark, and various districts of the United States of North America, *e.g.* to New York, Baltimore, St Paul, Minn., and St Louis, in the more northerly part, and to New Orleans which represents the southern climate. Some of the observations thus made have already been published, while others have been privately communicated.

In *Great Britain*, careful experiments were carried out by Sir Felix Semon⁽²²⁾ in London, and Dr McBride⁽¹⁸⁾ in Edinburgh. The first-mentioned made observations both with toxin and antitoxin on five hay-fever patients and seven control subjects, with results corroborating Dunbar's. Two of the controls, it is true, also reacted slightly to the toxin. One of these suffers from asthma, and is hypersensitive to wind and dust, while the other says he is liable to sudden violent paroxysms of sneezing, with considerable rhinorrhoea, especially in the early morning at any time of the year.

Dr McBride experimented with four hay-fever patients. Three of these gave very typical reactions to the toxin. The patient who did not react suffers from similar attacks in winter. There was no reaction in the two control subjects. These results are also borne out in communications from the other sources, where all the cases tested reacted to the toxic pollen and testified to the efficiency of the antitoxin, both as regards neutralising the toxin *in vitro*, and cutting short attacks artificially excited. Control subjects were used in all cases.

All the cases experimented with reacted to the toxic pollen: a few showed no reaction to the toxin itself. This had also been observed in laboratory experiments with dilute solutions of toxin. On increasing the strength of the toxin, however, typical symptoms were produced. It is therefore extremely probable that outside cases would have also reacted to a toxin less dilute or more fresh. Quite recently several eminent specialists in Hamburg have privately communicated the information to us that the results of their experiments with a number of hay-fever patients confirm Dunbar's view of the etiology.

Of great interest is a letter from a doctor in Egypt, who, while engaged in collecting maize pollen, visited a friend who suffered regularly at the time when maize flowered there from persistent 'cold in the head,' especially marked when he made railway journeys. He had not been with him many minutes when his friend developed a perfectly typical attack of hay fever. On another occasion to put the matter to the test this gentleman snuffed some maize pollen up the nose. After a few seconds he was attacked with severe sneezing, and an intense catarrh, with swelling of the nose, profuse lachrymation, and smarting in the eyes. These symptoms lasted three days, and were so bad as to prevent his sleeping at night. No antitoxin was available, and the patient could not be induced to repeat the experiment! He also tried the effect of maize pollen on three control persons, who experienced no symptoms.

Some of the results obtained in *America* have been published by Mayer⁽¹⁰⁾ of New York. These results, and the others found in the correspondence, make it very probable that the early spring form in America, the so-called 'June cold,' is identical with typical European hay fever, and is produced by the pollen of grasses.

Autumnal catarrh is apparently the most common form of the disease there, and, as has been mentioned, is popularly believed to arise from the pollen of such late-flowering plants as 'Golden Rod' and 'Wormwood.'

Of all American cases on which pollen and toxin were tested only three suffered from the early form of the disease, the 'June cold.' Two of these gave typical reactions to maize pollen toxin. The third showed no irritation. It is probable that the toxin used in this case was not fresh.

In a number of cases of autumnal catarrh experiments were made with toxin obtained from maize and from barley. Most of the cases were insusceptible to these toxins. One was irritated by the barley

toxin. All of the cases reacted which were tested with *Solidago* (Golden Rod) pollen, and toxin respectively.

Antitoxin (obtained by means of pollen of plants belonging to the Gramineae) was used in six cases, always with prompt beneficial result.

Autumnal catarrh, therefore, differs from European hay fever only in not being excited by the pollen of the grasses, but instead by the pollen of *Solidago* and *Ambrosia*, and possibly other late-flowering plants. These patients pass the early summer free from all symptoms, and only show signs of the disease when autumn appears. That this autumnal catarrh has a close relationship, however, to European hay fever is shown by the fact that the symptoms of the disease can be subdued by the antitoxin for the pollen of graminaceous plants.

It is evident from the foregoing that a toxin isolated exclusively from the pollen of certain plants is able to call forth in hay-fever patients, in all civilised countries, independent of the season, typical attacks of the disease. Conversely, we may conclude that hay fever as it occurs in the different civilised lands is really an etiologically identical disease, so far as the exciting cause is concerned. What it is that brings about the hay-fever predisposition is a subject of much greater difficulty, and will not be entered upon in this paper.

So constant have been the reactions shown by hay-fever patients to pollen toxin, that this toxin may have a distinct value as a *diagnostic agent*. Especially valuable will it be to the practitioner in discriminating from hay fever such cases as those of coryza nervosum, and many catarrhal ailments closely resembling hay fever except in its peculiar periodicity. Cases of pure asthma could by this means also be differentiated.

Such observations with pollen toxin have already been made, and this use of it recommended to the profession by one or two specialists, including Dr Thost of Hamburg.

It is now proposed to consider in how far treatment by pollen antitoxin is likely to be an important therapeutic advance on existing methods of treatment of hay fever.

Everybody admits that treatment in this disease has been up to the present time unsatisfactory. Many therapeutic methods have been proposed and introduced, with in certain cases apparently some measure of success. Such success however has usually been of a partial nature, or the method of treatment has been limited in application only to certain classes of patients. As is shown by very general practice the

great majority of sufferers find their only safety in flight to the sea or sea-coast, or away to certain mountainous regions where they remain until the hay-fever season is gone by. In these localities they can usually spend the days out of doors without using any of the remedies recommended. Of such remedies cocain has had a long trial, and is generally thought to be of most benefit. In this disease however cocain becomes a dangerous drug to use, on account of the frequent necessity for reapplication and the very common tendency of the patients to neurosis. In a very large number of cases cocain is found to be useless. Quinine is recommended by some: adrenalin has been much advocated by others. Morell Mackenzie found good results, he said, from insufflations into the nose of a powder consisting of a sixteenth of a grain of morphia and one grain of bismuth. More heroic treatment has been recommended. Cazenave was the first to cauterise the mucous membrane of the nose by way of lessening its susceptibility to hay fever. He used silver nitrate for the purpose. The electro-cautery has for the same reason been much employed.

The new treatment, founded as it is on a rational etiological basis, should, theoretically, provide a cure for all the symptoms of the troublesome malady, in all cases of the true disease. Hay fever being a disease arising in the predisposed from pollen toxin being inhaled by the patient from the air, if this toxin is neutralised before it has time to set up local irritation, or has entered the circulation, attacks of hay fever *should not arise at all*. An examination therefore into the results obtained from the use of the remedy in actual practice during last summer,—the first season of its introduction,—will be of the greatest possible interest.

PART II.

The Results of Prevention and Treatment of Hay Fever by means of Pollen Antitoxin during the Summer of 1903.

Through the courtesy of Prof. Dunbar the publications and private correspondence dealing with the general experience of doctors and patients in the summer of 1903 in regard to the value of the antitoxin were placed at my disposal, and I have been able, having critically examined these, to arrive at an estimate of the practical results of prevention and treatment in cases of the natural disease.

Before proceeding to an actual discussion of cases a few notes on the antitoxin will be given, with regard to its preparation and to the proper method of its use.

Further Notes on the Preparation of Pollen Antitoxin.

In sending out the serum, the greatest care was taken to ensure its sterility, the entire absence of all toxic properties, and the constancy of its antitoxic value.

1. The horses used for obtaining the serum were always under the inspection and control of a veterinary surgeon. Healthy horses were in all cases used, and the blood was withdrawn from them under aseptic precautions, and the after-manipulations with the serum were carried out with strict attention to asepsis. In order to make certain that no undestroyed pollen toxin remained in the serum, no blood was withdrawn from the horses until from six to eight days after they had perfectly recovered from the effects of the last injection of pollen toxin, and until they had regained their former weight. These effects after injection consisted in fever, loss of appetite and weight, and in local swelling. The serum was regularly tested to ensure sterility, and received an addition of $\frac{1}{4}\%$ carbolic acid as preservative.

2. That the serum should always have a constant antitoxic value it was regularly standardised, and its power to neutralise pollen toxin estimated.

The method used for standardising serum is as follows:—Prof. Dunbar and Dr Prausnitz, both hay-fever patients, use the reaction of their conjunctival mucous membrane for the purpose of testing the strength both of toxin and antitoxin. The procedure is as follows:—In the first place a solution of toxin is chosen which just causes an unmistakeable and typical reaction. Such a reaction is observed after a few minutes, and consists in subjective itching and feeling of heat, and objective reddening and swelling of the caruncle, plica semilunaris, and eyelids, of the eye treated. There is also injection of the limbus corneae, and of the conjunctival vessels. Then a series of mixtures of toxin and antitoxin are made, each containing that minimum toxic dose described above, along with decreasing quantities of antitoxin. The amount of antitoxin present in that mixture which just gives rise to no subjective or objective reaction is taken as a measure of the antitoxic strength. Frequently repeated experiments with this minimum toxic dose of pollen toxin show no marked decrease in the susceptibility of

the conjunctiva to this standard. Trials of other methods for standardising the serum are being made, but the one given has so far been found the most dependable. The antitoxic value of the serum does not apparently markedly diminish even after the lapse of several months.

The serum treatment of hay fever last summer was almost entirely confined to local applications to the conjunctival and nasal mucous membranes. The preparation used by patients was at first a fluid serum to which $\frac{1}{4}\%$ carbolic acid was added as preservative. It was sent out in a sterile condition in small glass tubes each containing 2 c.c. An empty glass phial with a dropping pipette was also provided, so that it was only necessary to use a small quantity of the serum at a time. In spite of this precaution and of the addition of $\frac{1}{4}\%$ carbolic acid the preparation very easily decomposed, and might be fit for use only for a few days after opening the tube. This decomposition was brought about by the frequent infection of the serum with germs from the air and from the nasal mucous membrane. A higher percentage of carbolic acid could not be used, as stronger concentrations gave rise to an unpleasant burning sensation in the eye; indeed a few particularly sensitive patients cannot bear even the $\frac{1}{4}\%$ addition, complaining of severe irritation in the eyes and nose from the carbolised serum. On the other hand the addition of other antiseptics less irritating to the mucous membrane was for other reasons found to be impracticable.

This decomposition does not always occur, and can to a great extent be prevented by only using a small quantity of the serum at a time, and by carefully cleaning and sterilising the dropping pipette after use. When decomposition does set in it is evidenced by a uniform turbidity, and sometimes by the serum developing an odour. This of course renders the preparation unfit for further use. To be distinguished from this uniform turbidity is a slight flaky deposit in the serum, that is often present on despatch of the remedy in sterile condition. It arises from the carbolic acid present.

On account of the ease with which the fluid serum decomposes, and the sensitiveness of many patients to carbolic acid, Dunbar sought to produce a preparation that would obviate these difficulties, and be more convenient in application and surer in result. For this purpose he dried the fluid serum, and obtained a powder, that without the addition of any chemical preserved well for a long period. The drying took place in a large vacuum apparatus at a temperature of 40–45° C. The dried mass was finely powdered, mixed with sterile milk sugar, and strained through a very fine sieve. The resulting preparation was a

fine white, almost inodorous powder. All the operations were conducted under the strictest aseptic precautions, and bacteriological examinations which were regularly carried out guaranteed the sterility of the preparation.

Method of use of Serum.

The fluid serum is chiefly suited for local applications to the eye. By means of the pipette a drop of 'Pollantin,' as the preparation is called, is brought to the outer angle of the eye and allowed to touch the mucous membrane of the lower lid, which has previously been drawn down by the finger. A pleasant cool sensation in the eye testifies to the operation having been correctly carried out.

For the nose, the serum is best used in powder form. It is sniffed up each nostril in small quantity. The contained milk sugar lessens the irritation of the powder, and gives rise to a slight secretion of nasal mucus which helps to dissolve the dried serum. The advantage of using the remedy in powder form, in addition to its convenience in application, is that it enables the serum to be spread more efficiently over the whole inner lining of the nose, and to reach the recesses in the mucous membrane and the apertures of the neighbouring sinuses. Immediately after sniffing up the powder the taste of the milk sugar can frequently be appreciated, showing that the powder reaches even to the throat. The powder therefore reaches at least as far as the pollen that has been inhaled from the air and thus possesses a very distinct advantage over the fluid preparation.

Should it be desired to use the powdered serum also for the eye, a small quantity is applied to the conjunctival sac by means of a fine sterilised camel-hair brush. There is at first the feeling as of a foreign body in the eye which however passes off in about ten seconds and the beneficial effect follows very quickly. Many patients who were apparently not at all, or not sufficiently influenced, by using the fluid serum were able to keep themselves quite free from attacks by help of the dried preparation.

Especially in combating hay asthma was the dried serum found to be of great advantage, for it reached better the deeper air passages and was also better absorbed. In most cases the fluid serum only succeeded in reaching the front portion of the nasal mucous membrane, and was soon expelled from there by the profuse nasal secretion.

In studying the best means to use pollen antitoxin in the treatment

of hay fever the well-known fact found in practice with other sera must be borne in mind, that when antitoxin is brought into use, after the toxin has had time to enter into combination with the body cells, a hundred, or even a thousand times the amount sufficient to neutralise toxin *in vitro* may be quite useless in treatment. It is therefore clear that the greatest importance must be attached to the prophylactic use of the serum. For this purpose patients were recommended to sleep during the hay-fever period always with their bedroom windows shut, and to apply the 'Pollantin' regularly every morning a few minutes before getting up, both to the eyes and to the nose. By this means, according to experience, a patient can guard himself from attacks for several hours, often indeed for the whole day. Exposure in the open air should be limited to a few hours at most. It was further recommended that serum should always be used before going out into the open air, and before exposure to a probable great amount of pollen infection, as for instance before railway journeys. If in spite of such precaution the beginning of an attack be noticed, with slight itching or burning in the eyes or nose, serum should be at once reapplied, in order to check the further spread of the process by neutralising the pollen toxin so long as it remains local. It is perfectly obvious that a good result cannot be expected from use of serum when the process has been allowed to go on till a severe nasal attack has set in. The nasal mucous membrane then becomes so swollen and the secretion is so great that serum can only with difficulty be applied, and is at once washed out by the great flow of watery mucus. Toxin has also entered into the circulation, and into combination with the body cells, enormously increasing the difficulty of serum treatment. In the treatment of such an attack, especially if asthma symptoms be also present, it is recommended that the patient should retire to rooms with doors and windows shut, and remain there until the symptoms have subsided. This will be hastened by using serum locally, at first every 10 minutes, and afterwards at longer intervals. When the attack has passed off, the patient should endeavour by such prophylactic use of the serum as has been described to prevent further attacks from appearing. The use of pollen antitoxin is not followed by any ill-effects, nor does it create a habit. On the contrary longer use imparts a certain slight immunity to the hay-fever poison, rendering the need for frequent applications less.

It was thought possible at first that by the use of subcutaneous injections patients might be passively immunised against hay-fever

toxin, but the results with this method prevented its recommendation. Experiments made by Dunbar and Prausnitz⁽¹⁰⁾, injecting hypodermically 1—2 c.c. of serum, certainly showed a favourable result in lessening the severity of attacks for three or four days. There was found particularly a distinct decrease in the local subjective symptoms, and in the general feeling of malaise. Immunity to the toxin, it is true, was not attained through the relatively small amount of serum used, but natural attacks of the disease, or attacks artificially excited by means of pollen, were in consequence of much less intensity, and more easily allayed by antitoxin locally applied. Similar results were observed by Borrowman⁽⁵⁾ in two cases where the injections were repeated, in the one case thrice, and in the other four times. Other doctors also in spite of Dunbar's warning appear to have employed this method.

This warning against the use of subcutaneous injections was given, because in most cases there appeared at the site of injection four or six days after, a swelling with erythema and much itching, and often an urticaria-like rash. These appearances usually lasted about a week and then gradually disappeared.

Sir Felix Semon⁽²²⁾, in reference to these after-effects, said, that it was obvious to him that the antitoxin as at present used must still contain toxic or septic properties. When he wrote this he must have been unaware of the strictness of the precautions taken, as previously described, to ensure that no pollen toxin remained undestroyed in the serum, thus preventing the possibility of the serum in use containing toxic properties. Careful testing for sterility in every case also precludes the possibility of the serum's possessing septic properties. When the serum was 'pasteurised,' or exposed to a temperature of 60° C. for half-an-hour, it caused the same effects after subcutaneous injection as serum not so treated. In not one of the many hundred laboratory experiments with local applications did the serum cause the slightest irritation to the mucous membranes of eye or nose. Irritation is occasionally noticed in a few hay-fever patients during the time of its prevalence when serum is applied to the sensitive mucous membrane of the eye in the course of an attack, but this effect is also produced by the application of any other remedy.

Although therefore these after-effects of subcutaneous injection appear to be quite without any dangerous significance and to be similar to those serum exanthemata observed after injecting other sera, *e.g.* diphtheria antitoxin, yet on their account Dunbar felt it inadvisable

to recommend the method. Could these irritating properties be removed the method would be one especially applicable to the treatment of asthma symptoms, but in the meanwhile it is satisfactory to know that local applications of the serum when properly used suffice to keep any patient free from attacks of the disease. A consideration now of the cases will show that the prophylactic use of the serum was followed by most excellent results in a great number of instances. A few found difficulty in the treatment at first, and only got a satisfactory result after obtaining the dried preparation without addition of carbolic acid.

Results of Serum Treatment in European Hay Fever.

	Positive results 127	Partial results 71	No improvement 24
Result in cases complicated with asthma }	14	6	9
Result of serum treatment in %	57 %	32 %	11 %

Cases.

The cases will now be cited in some detail, and are arranged in the order of the results obtained. The cases of the disease in America have been reserved for a special description at the end.

The total number of patients who during last summer sent reports of their experience with pollen antitoxin amounts to two hundred and eighty-five. The cases are representative, as the patients reside in very different countries, where different forms of the disease might be expected. Reports are to hand, namely, from England, Scotland, Germany, different parts of Austria, France, Holland, Denmark, Russia, Switzerland, Italy, and America.

Excluding reports from the last-mentioned, the communications deal with 222 hay-fever patients. When it is stated that a completely successful result followed the use of the serum in 127 cases representing 57 % of the total, it will be agreed that such a result, considering the difficulties and misunderstandings always present on the first introduction of such a remedy, is both satisfactory and highly encouraging. In 71 cases, that is in 32 %, a partial result was obtained. 24 patients or 11 % were unsuccessful in obtaining any benefit from its use. 171 patients,

which is a majority of the cases, found it relatively easy to keep themselves free from attacks by its means.

Cases where Complete Results were obtained by Treatment.

A few typical case-histories will be given in detail.

Case 1. Dunbar has already published the case of a young officer, a great sufferer from hay fever which made him dread the manœuvres, when his duty compelled him to be actively employed during the hay-fever period, in the open air amongst flowering cornfields. During last year he had also important work on hand necessitating his being in good health. He was able by using a drop of *fluid* serum every morning before rising, in each eye and nostril, to keep himself free of all irritation for four or five hours at a time, even at the height of the hay-fever season. In the second half of June he was able after using serum on one occasion to keep all attacks in abeyance for nearly two days except that he sneezed six or seven times, in the morning and evening. He said that he had never before come so easily through the hay-fever season.

The following communication from a doctor in Görlitz is given in full.

Cases 2—11. "I had the opportunity of treating ten patients with the serum without a single case of failure. I followed the accompanying directions carefully and strictly and obtained a very excellent result in five of the patients, whose eye symptoms were more troublesome than the nasal. In the case of two of these patients (both officers) for several days in succession two drops of serum were instilled into each eye, in the early morning before they went on duty, and they both agreed in reporting immediate relief from the burning in the eyes, and that they were able to take part in exhausting manœuvres in dust and sunshine, which was formerly quite impossible for them. The effect of the serum passed off after twenty-four hours, sometimes earlier: it lasted longer in cool than in hot weather. In the case of the other patients the excessive secretion from the nose and eyes immediately diminished. A lady very sensitive to the hay-fever poison found that the effect of the remedy only lasted a few hours (five to six). With three others it lasted even in sunny weather for three or four days."

The following is the case-history of a lady, in whom the effect of the serum was carefully observed by her family doctor.

Case 12. Lady in Stettin, Germany, 30 years of age, is otherwise perfectly healthy. She is in no way hysterical, a woman of sound judgment and reliability. Outside the hay-fever period she is able at all times to fulfil her social and domestic duties. For many years past patient has suffered from hay fever, which, coming on at the end of May, lasts several weeks, during which time she is quite unable to do anything. The conjunctival and nasal irritation is so severe as to cause her much suffering. Three or four years ago the hay fever was so bad that for some time she required to remain in bed on account of diffuse bronchitis, with asthmatic symptoms.

Experience with the serum. Fluid serum was used. First attack in 1903 was on May 29th. Between the 3rd and 5th June the conjunctival blood vessels had become intensely injected, and the nasal mucous membrane was red and swollen. After instillation of the serum the patient found subjective alleviation, and the objective signs disappeared. She felt "as though the serum prevented an extension of the process further than the throat."

After three days, during which 2 c.c. serum had been used which had kept her free from attacks, her stock of the antitoxin was exhausted. She felt the want of it very keenly.

Since using the serum she can go out of doors even in the worst days of the hay-fever period, and she maintains that she not only got immediate improvement by its use, but that on those days when she did not use it a certain beneficial after-effect was perceived keeping the illness in check.

Cases with Positive Result after initial difficulty with the treatment.

Still more instructive are the case-histories of those patients who experienced at first considerable difficulty in obtaining a satisfactory result through serum treatment, but later on succeeded in protecting themselves from their attacks by its use. This preliminary difficulty is to be explained either by an unusual susceptibility of the individual to the carbolic acid contained in the fluid serum, or probably in most cases by incorrect use of the serum, the patients not carrying out the important directions for prophylactic treatment.

Case 13. Doctor, 41 years of age, in Amsterdam.

Has suffered severely from hay fever for 24 years and is usually rendered almost incapable of work from the middle of May till the end of July. He is affected alike in town and country, and has attacks even in the house, where doubtless as he himself says pollen grains are brought in by clothes, newspapers, etc.

In treatment he has not found much benefit from former remedies. He tried without success antifebrin and quinine internally and local applications of adrenalin and cocain, also treatment by the galvano-cautery.

Experience with the serum. His first attack in 1903 was on 25th May at 9.30 a.m. At 10 o'clock he instilled five drops of serum into each nostril, and one or two drops into each eye. Result in three minutes absolutely successful. The effect in the nose lasted till 2.30 p.m., when a fresh nasal attack was aborted by five drops of serum in three minutes. In the course of the next few days he found that instillations once or twice a day sufficed to keep him free from attacks. On the 28th May he required no serum. The eyes were always easier to keep in a normal condition than the nose.

In the beginning of June he found that he obtained no relief from attacks of his hay fever by use of serum. At the same time he was also suffering from an acute attack of rhinitis, and while that lasted the hay-fever attacks were unchecked by the antitoxin, although lessened in severity.

The "cold" passed away with the use of Salipyrin. On the 9th of June patient had a pure attack of hay fever. The day was warm and an east wind was blowing. The cold in the head was by this time gone. On this day cocain was tried, but gave no material benefit. After the use of the antitoxic serum he was in half a minute quite free from symptoms.

Dried serum was sent to patient on the 23rd June. Since then he has been able, by the constant use of this preparation, to keep himself continuously free from all attacks. He wrote repeatedly to say that the dried serum had most excellent results; usually it was only necessary to employ it in the nose, although in severer attacks it was applied to the eyes as well.

Case 14. Doctor in Jena, 54 years of age. Has suffered from severe hay fever and asthma since 1867.

At first the irritation is limited to the conjunctiva, then it spreads to the mucous membranes of the nose and deeper air passages, giving rise to asthma. There are also symptoms referable to the stomach and rectum. He suffered so severely in 1870 that marked mental depression supervened. He describes the asthma attacks as being particularly severe, but the condition in the eyes and nose was at times almost unbearable. He noticed that after severe attacks there often followed periods of rest which might possibly be ascribed to active immunisation. Treatment formerly by introducing water into the nose gave him some slight relief. Cocain was not of much help to him. Formalin gave rise to great irritation.

Experience with the serum. In 1903 his hay fever began on 28th May, and the first slight attacks were successfully treated by instillation of the antitoxin. On the following days the patient journeyed from South Germany, through the Harz to Heligoland. Several attacks occurred on this journey, which by use of serum were either cut short or improved. On 4th June while climbing the Brocken he had a severe attack. On using serum he noticed that there was marked diminution in the vascular injection of his conjunctivae. This was confirmed by two other doctors.

On the 9th June, on a sailing cruise to Heligoland, he had a severe attack. Another hay-fever patient on board was affected at the same time. Instillation of fluid serum into his eye was followed by immediate improvement. This improvement was less marked in the nose, but the nasal hypersecretion was distinctly lessened. The patient was not, however, able to keep himself continuously free from attacks by means of the fluid serum, and even with the dried serum he was unable at first to successfully combat the disease. On the 19th and 20th, which were hot, windy days, patient was comparatively free from hay fever, although he had slight asthmatic attacks. After the 21st June he found more benefit from the antitoxin. On that day he sniffed powdered serum up the nose, and also introduced some into the eyes. During the day he was exposed to much pollen infection, having to stand for two hours under a hot sun and remain the whole afternoon in the open, and danced in the evening, yet had no attack and passed the next day also without attack, only having a slight increase of nasal secretion until the evening, when a fresh attack was immediately cut short by the powdered serum. On 27th June, when the hay-fever season was still at its height, he wrote that since he decided to sleep with windows closed and use the serum immediately on waking in the mornings he had been quite free from attacks, except on two dry, hot, and windy days, when he had not used the remedy at the proper time.

Besides these cases given in detail, there are communications from eight other patients, all persons of unusual susceptibility to the pollen poison, whose cases illustrate this same initial difficulty and final success from prophylactic use of the serum. An interesting experiment on two of these patients may here be described.

Accompanied by a doctor, not subject to hay fever, a railway journey was made to a place in the neighbourhood of Hamburg, where there was much corn and grass in flower. The day was sunny, with a slight wind. Patient *A* used serum prophylactically and repeated its use on the appearance of the slightest irritation. Patient *B* used no serum. Patient *A* remained continuously free from attack, only requiring to sneeze once during the excursion. Patient *B*, on the other hand, suffered from repeated severe sneezing fits, was incapable of work in the evening, and still felt the effects of hay fever on the following day.

On 24th June the same tour was again made, but this time patient *A* used no serum whereas patient *B* did. On this occasion patient *B* was able to completely protect himself from attacks, as did also another hay-fever patient who took part in the excursion and used serum. Patient *A*, however, suffered on this day from sharp eye and nose attacks, and was in a very miserable condition in the evening.

It is of great importance to know that the pollen antitoxin has been effectual in combating even the severest symptom of hay fever, viz., the asthma attacks. Hay asthma can apparently be excited by the local action of toxin from pollen grains which have reached by deep inhalation the bronchial mucous membrane, or it may arise from toxin absorbed by the mucous membranes of eye and nose and circulating in the blood.

It will be remembered that asthma resulted from a deep inhalation of pollen, in the case of the experiment with a hay-fever patient in the glass chamber. Similar asthma-like attacks resulted in a hay-fever patient from unintentionally breathing in powdered pollen toxin in the laboratory.

Further, it is known from earlier experiments that after subcutaneous injection of an extremely small quantity of pollen toxin asthma resulted, showing that toxin circulating in the blood may excite an attack of asthma. We may therefore suppose that natural hay asthma may, in many cases, result from a toxin absorption from pollen on the nasal mucous membrane. Such a toxin absorption probably explains those general symptoms observed by every hay-fever patient, such as depression and irritability, lassitude, sleeplessness, fever, etc. This points to the necessity in treatment of neutralising the pollen toxin before it can be absorbed into the blood.

Results in treatment of asthma cases.

The following histories show the influence of the antitoxin on cases with asthma.

Case 15. Postman, 38 years of age, living in the Harz mountains. Suffers from severe hay fever, with bad asthmatic attacks. Since 11th July he used dried serum daily, morning and evening, applied to each nostril and to his conjunctivae. All symptoms disappeared after a week's treatment. The result of treatment was afterwards complete.

Case 16. Patient suffers every year, from end of May onwards, from attacks of sneezing, conjunctivitis and tickling sensation in the ears. Attacks in 1903 were at first slight, and were benefited by adrenalin. After a walk in fine weather in the neighbourhood of Vienna amongst meadows a severe attack in eyes and nose appeared, which obstinately continued for several days and nights. At this stage fluid serum was tried. After 1 c.c. had been used all symptoms of asthma and hay fever passed away, and the patient's medical man reported the result as a very successful one.

Case 17. Lady, 25 years old. Suffered for several years from severe hay asthma. All possible remedies had been previously unsuccessfully used. Only by resorting to a locality on a higher elevation where grass flowered at a later date could she in former years keep attacks away. Last year energetic treatment with pollen antitoxin was begun, soon after the commencement of the hay-fever attacks. Within a day and a half all symptoms were gone, and the patient remained free of attack, till, after a long bicycling excursion amongst flowering crops, an attack set in, but only of moderate severity, which was soon checked.

Since then patient succeeded in keeping herself free from all attacks without being compelled to keep the house.

Naturally such results with asthma symptoms were not always obtained, and indeed it was very frequently found that hay-fever patients succeeded in checking by use of serum all their symptoms with the exception of the asthma. Such cases belong really to the group of partial results, but a history may be cited here in detail in order to complete the discussion of asthma.

Case 18. Middle-aged male patient, has been affected for 15 years, from end of May onwards, with hay fever and severe asthma. By instilling fluid serum twice daily patient could almost completely get rid of his nose symptoms, whereas, in the previous year, he often had to sneeze twenty or thirty times in succession, and suffered from very profuse lachrymation. The asthmatic attacks, however, were about as severe as they had been in the previous year, hindering him from working, and making even the simplest actions, such as eating, getting up out of bed, etc., a task.

The reason for this lack of success is not apparent from the communication. The following table gives statistics with regard to treatment with serum in asthma cases.

Detailed results of Serum Treatment in Hay Asthma.

Method of application	Positive result	Partial result	No improvement
Subcutaneous injection	0	0	1
Fluid serum nasal	8	6	5
Dried serum nasal	6	0	3
Total	14	6	9

Cases where complete benefit was not obtained.

The last leads to a discussion of the second group, viz., those who obtained only a partial result from treatment with the pollen antitoxin.

Seventy-one cases are reported belonging to this category, that is 32 % of the cases treated.

Agreeing with Lübbert and Prausnitz⁽²⁹⁾ I have arranged all those cases of hay fever in this group:

(a) who reporting success with the treatment sent very incomplete details of their cases,

(b) who gave only a preliminary report of success at the beginning of the season, but sent no further communication,

(c) who obtained a successful result with some of their symptoms, but others were not influenced, *e.g.* eye irritation was allayed but not nasal symptoms, or *vice versâ*, or, as was more frequent, eye and nose attacks were checked but asthma remained,

(d) where light or moderately severe attacks were cut short by serum, but where such result was not obtained in attacks of greater severity.

The first and second classes may indeed contain many perfectly complete results, but could not be so used in statistics, since these statistics are based on results obtained throughout the hay-fever season, and not on the effect of serum on single attacks.

In the next class are found those patients who obtained relief during attack from certain symptoms but not from others. The reports of such cases show that as a rule there was an unsatisfactory lack of system in the method of carrying out the treatment, and very frequently an utter disregard of the prophylactic use of the serum. About 68 % of these patients also only used fluid serum.

The eye symptoms were generally found to yield more easily to treatment than the nasal ones. This is explained by the much greater difficulty found by most patients in correctly applying the serum to the nose.

Two examples, besides the case described when discussing asthma, will suffice as illustration of this class.

Case 19. A young doctor in Silesia wrote :

"The serum had always a good influence over the affection in the eyes. After injection of one drop the burning almost immediately diminished, and only returned after some hours. The increased vascular injection was not markedly influenced by its use. The result in regard to the sneezing fits was practically absolutely negative. For a short time, perhaps five or ten minutes, there was felt a pleasant cool sensation with dryness in the nose. Sneezing ceased, but commenced again with undiminished force, and the nasal secretion also came on to the same extent as before.

On the whole I sincerely welcome the serum as an efficient remedy, for even the removal of eye symptoms is a great benefit, and is more than any other remedy has hitherto been able to accomplish."

Case 20. A doctor in Holland wrote :

"The itching in the eyes disappears on instillation of pollantin wonderfully quickly. The drop at once gives a pleasant cool feeling in the eye. I have not noticed much effect in the nose. At first the sensation is not unpleasant but the secretion is increased, and on the evening of the first day I candidly felt myself very unhappy. The attacks diminished somewhat in severity after a few days."

The next class of cases with partial result includes those who found relief in slight or moderately severe attacks, but where severe attacks were uninfluenced.

From what has been said in the introduction to this discussion this experience is not to be wondered at, and is generally found in practice with other sera, such as diphtheria or tetanus antitoxin, where severer intoxication from the respective toxins is practically unaffected in treatment with antitoxin. In this case pollen antitoxin has not got the chance of influencing severe attacks, not only on account of the physical difficulties in its application, but also because the toxin is already in the blood stream and has entered into combination with the body cells. The right method of treating such attacks has already been dealt with.

Case 21. The following case has already been published in the *Geneeskundige Courant*, 1903, No. 28.

Lady, wife of a doctor. She has regularly suffered from typical hay fever for seven years. In the past year it was associated with asthma. In the hay-fever period she frequently suffers from urticaria on both hands and forearms.

After three weeks' observation the doctor came to the following conclusions :

Slight and moderately severe attacks were always promptly cut short by means of fluid serum. To subdue slight irritation in the eyes or nose, one drop of serum sufficed and effectually checked the development of an attack. Larger doses were

required in attacks of moderate severity. The convulsive sneezing was likewise stopped by instillation of a small quantity of the serum in each nostril.

If after exposure to much pollen infection a severe attack ensued, so that the eyes were inflamed and the nose much swollen, then serum had little influence. Its application was rendered next to impossible by the great amount of watery mucus secretion.

Case 22. Another instance is that of a young man, a sufferer from hay fever for years, who had tried without benefit different methods of treatment, recommended from year to year. His family doctor reported that use of the serum was followed by such marked result that the patient thought that he was for ever cured. On a journey to a hilly district severe attacks again appeared on which the serum had no influence, obviously because of the severity of the pollen intoxication.

Cases where no benefit was obtained by serum treatment.

The last group contains the cases with entirely negative results. Of these communications four cases have not been included, since from their histories they were manifestly not true cases of hay fever. On the other hand there have been included all those cases who from the manner in which they used the serum could not expect any result to follow. This group contains 24 cases, or 11% of the total.

As it has been said, many of these used the serum in an irrational manner, this was practically demonstrated in an instance where the patient was asked by Dunbar to show how he applied the remedy. He put some powdered serum on the back of his thumb, and approaching it to his nose scattered it to the winds in his effort to sniff it up. Yet he imagined he had applied the preparation properly, and had shown its uselessness in an attack. When a few minutes later serum was really introduced into the nose the symptoms of his attack disappeared.

Other patients used the antitoxin far too seldom. A lady from Dundee wrote that she had thrice used fluid serum in the course of three weeks and could not say that she had noticed any marked result.

Quite a number of patients would not carry out the method recommended for prophylactic treatment, refusing to sleep with windows closed. Hay-fever patients have a feeling in the night of the necessity for fresh air, but sleeping with open windows they do not give the sensitive mucous membranes that period of rest from the influence of pollen toxin which they require. By separating out those cases where according to their statements one of the foregoing reasons can be given in explanation of their failure with the serum, 9.6% can be deducted from this group of negative results, leaving only 1.4% of cases where

no reason for this result can be found. Possibly they would have found, as others did, that the powdered serum was more efficient.

From this review of the treatment of European hay fever with pollen antitoxin last summer, it will be seen how very encouraging the results are, and that they give reason for the belief that when all patients learn for themselves the rational prophylactic use of the serum, and carry out all the precautions necessary in their individual cases, they will be able to pass through the hay-fever period without anxiety.

Benefit from serum therapy in complicated cases.

If this is a matter of importance to ordinary hay-fever patients, how much more will physicians welcome a means of warding off hay-fever attacks in patients where they form a serious complication of other conditions. Information is to hand for instance from a doctor who treated successfully with serum a case of hay fever in a markedly neurasthenic subject, and by this means greatly improved the nervous symptoms. A similar result was obtained in the case of a lady with psychical disturbance. Several cases of the beneficial effect of serum in patients with arterio-sclerosis are reported, where the strain of the sneezing fits or asthma attacks was naturally a great danger. Also the following case is of unusual interest in this connection.

Case 23. Pastor in the country had formerly catarrhal affection of the apex of the left lung, suffered in summer, 1903, from severe hay-fever symptoms. These symptoms consisted of enormous nasal hypersecretion with marked hyperaemia of the nasal mucous membrane. Nose completely blocked to the passage of air. Severe conjunctivitis, and such general symptoms as malaise, lassitude, slight fever, loss of appetite, and especially important a lighting up of the lung mischief in the left apex (Râles medium, sputum mucopurulent, tubercle bacillus not detected).

Instillation of fluid serum according to directions in eyes and nose.

Improvement in nose very soon after, and the passage of air through the nostrils became once more free. The lung catarrh rapidly healed. Patient did not treat the eyes with serum, but with a solution of cocain and zinc, as he found serum in the eyes unpleasant.

Results in America.

Attention will now be turned to the experience with serum treatment of hay fever in America. It has been already mentioned in discussing the etiology of the disease that hay fever occurs in America in two forms, viz. 'Spring cold' and 'Autumn catarrh,' and that the

latter differs from European hay fever only in so far as it is excited by the pollen of *Solidago* and *Ambrosia* instead of by grass pollen.

For this reason and from the fact that in consequence of experience already gained the results of treatment were there much better than in Europe, these American cases have been reserved for a special description.

A case of June cold, however, with asthma attacks, reported with successful result of serum treatment (dried serum) from St Louis, U.S.A., has been included in the previous statistics.

The experience of several American doctors with autumn catarrh has already been published. The first antitoxin sent to America was the European antitoxin obtained from rye and maize pollen, but later a special antitoxin was used, which was obtained after the injection of *Solidago* pollen into horses. This latter antitoxic serum could, it was found, be standardised by Dunbar, since his conjunctiva is also highly sensitive to *Solidago* toxin. Reports did not however show that this latter antitoxin possessed any marked advantage over the other.

MacCoy⁽¹⁷⁾ of Philadelphia describes his experience with fifteen cases of autumnal catarrh. One of these will here be cited in detail:

Case 24. "Patrick — has had hay fever for eight years, coming on in early August. Has not missed a season during the period. The patient has been under my care for six years, and has been treated each year according to the latest and best remedies, but with little relief. Owing to my absence from home during August the patient did not come under treatment until September 2nd. I found him suffering from a pronounced attack. After two days' treatment he had some relief from symptoms. The relief continued and increased, and at the end of the week all symptoms had abated, and he had complete freedom from sneezing, itching and watery discharge, and has no asthma, which in previous years had been especially bad, on or about September 10th. In previous seasons, the asthmatic seizures had been severe, and had lasted all through September and part of October. This case has been under daily observation, and has continued—and remains—absolutely free from every symptom. This patient used eight bottles of serum—about a bottle a day; much more than any other patient under my care."

He further says: "We have all heretofore experienced such deep disappointment in our trials of various methods of cure—surgical and medicinal—that the writer was, to say the least, not enthusiastic concerning results, but he can truthfully say that he believes that no such advances have ever yet been made in the treatment of hay fever."

The total number of American cases reported is 63. Although it may not seem a large number, yet since many doctors had each several cases (10—15 patients) the conditions of observation were more favourable. A large proportion of patients with asthma symptoms were successful in combating it through the serum, as will be seen in the

accompanying table. Such being of greater interest than others the review of cases will be closed by giving in detail the experience of two of them :

Case 25. Patient living in St Paul, Minn., U.S.A., suffers every year from end of May till end of September from severe hay fever, with asthma and loss of sleep. After this she is much prostrated and requires a lengthened stay at a health resort before she is well again. In 1903 the first appearances of hay fever began on 21st July. From 15th August onwards she used pollen antitoxin with strict regard to the instructions. Within a week she was completely free of asthma, and was able to stop treatment on 12th September.

Case 26. The last case is that of a patient who has suffered for a number of years so severely from hay fever that she required to keep her bed for the better part of six weeks. In 1903 serum came into use on the day following the appearance of the first symptoms, on the 18th August. By its use the patient was able to pursue her usual duties without discomfort and had very little asthma. She was able to leave off treatment after three weeks.

The general results and percentages of recovery after serum treatment in autumnal catarrh are given in the table below. It will be seen how satisfactory and promising they are. It is needless again to enter into a discussion of the partially positive and negative cases.

The same considerations apply to them as did in the case of our own hay fever, and it is to be hopefully expected that in this year the number of such results will, with a better understanding of the exciting cause of the disease, greatly diminish.

Results of Serum Treatment in Autumnal Catarrh.

	Positive result	Partial result	Negative
	44	12	7
Cases complicated with asthma	8	2	3
Result of serum treatment in %	70 %	19 %	11 %

Conclusions.

In conclusion, briefly summarising the chief points discussed in this paper, it will be noted that Dunbar has, by his researches, gone very far to prove that there is in reality but one exciting cause for hay fever, this being the pollen of grasses and of certain other plants. He found that these pollens could artificially excite attacks of hay fever when applied to the conjunctivae or nasal mucous membranes of persons predisposed to the disease, and this even outside of the hay-fever

period. The attacks were in every way similar to attacks of the natural disease. Dunbar tested all the theories previously advanced to explain the etiology of hay fever, but found them all unsatisfactory. He succeeded in isolating the peculiar poison in the toxic pollen, and found it to be an albuminous substance, so toxic that even '000025 milligrammes of the proteid body, consisting of this active albumin and also of inert globulin, could excite irritation in the conjunctiva of a predisposed patient. This amount of toxin would be contained in two or three pollen grains. Large doses produced very severe attacks, and toxin injected subcutaneously produced most unpleasant and indeed dangerous symptoms in two hay-fever patients. Yet potent though this toxin be in exciting hay-fever-like attacks in subjects predisposed to the affection it is absolutely without any effect on normal persons.

By injecting pollen toxin into animals Dunbar succeeded in obtaining an antitoxin, which neutralised the toxin *in vitro*, and cut short attacks of hay fever artificially produced by the toxin. Furthermore the antitoxin cut short attacks of the natural disease. The toxin and antitoxin have been tested by a large number of doctors and patients in different parts of the world, with results which confirm Dunbar's. The antitoxin appears to be quite harmless. Owing to the peculiar nature of the disease, and the constant reinfection of the mucous membranes by pollen on exposure in the outside air, Dunbar has found it to be necessary, in treatment, to use the serum prophylactically, to sleep with windows closed, apply serum in the morning before rising, both to eyes and nose, and again during the day on the appearance of the slightest irritation in the conjunctiva or nasal mucous membrane. By this means he has succeeded and others have also been successful in keeping absolutely free from attacks of the disease.

It is to be hoped that the value of antitoxic serum treatment will be yet more widely tested in the coming hay-fever period, and the results carefully recorded, in order that statistics drawn from a very large number of cases may be obtained and the question of hay-fever etiology and therapeutics be set upon a still surer foundation.

My best thanks are due to Professor Dunbar for his kindness in putting at my disposal the material from which this discussion and review of the recent researches in hay fever has been derived. I have also great pleasure in acknowledging my indebtedness to Dr Lübbert and Dr Prausnitz of the Hygienic Institute in Hamburg for much kind help in the work.

APPENDIX.

TABLE OF PLANTS.

Examined and tested on Hay Fever Patients by Drs Dunbar and Prausnitz for toxicity of Pollen, including all which have been up to the present found toxic. (Collected and arranged by Dr Kammann according to the Linnean Classification.)

Class	Order	Specification of Plants	Toxicity of Pollen	Class	Order	Specification of Plants	Toxicity of Pollen
I.		No plants obtainable		V.	1.	Verbascum nigrum	-
II.	1.	Syringa vulgaris	-			Convolvulus arvensis	-
III.	1.	Iris germanica	-			Viola tricolor	-
	2.	Secale cereale	+			Campanula rapuncu-	
		Avena sativa	+			loides	-
		Hordeum sativum	+			Verbascum phlomoides	-
		Avena flavescens	+	2.	Heracleum sphondylium	-	
		Oryza sativa	+			Coriandrum sativum	-
		Calamagrostis lanceo-				Daucus carota	-
		lata	+			Conium maculatum	-
		Calamagrostis montana	+	3.	Sambucus nigra	-	
		Calamagrostis Halleri-		5.	Linum usitatissimum	-	
		ana	+	VI.	1.	Lilium Harrisii	-
		Dactylis glomerata	+			Tulipa	-
		Poa pratensis	+			Narcissus	-
		Anthoxanthum odora-				Hyacinthus	-
		tum	+			Convallaria majalis	+
		Eriophorum vaginatum	+			Polygonatum multifo-	
		Cynosurus cristatus	+			rum	+
		Phalaris arundinacea	+			Scilla	-
		Lolium perenne	+			Luzula pilosa	-
		Holcus lanatus	+	3.	Rumex hydrolapathum	-	
		Alopecurus pratensis	+	VIII.	1.	Oenothera biennis	-
		Aira caespitosa	+			Erica	-
		Brachypodium sylvati-		XII.	1.	Prunus avium	-
		cum	+			Philadelphus coronarius	-
		Agropyrum repens	+	2.	Pyrus japonica	-	
		Festuca elatior	+			Spiraea ulmaria	-
		Festuca gigantea	+	3.	Geum rivale	-	
		Crocus	-			Rosa (7 different var.)	-
		Triticum sativum	+			Rosa canina	-
IV.	1.	Plantago lanceolata	-			Rubus fruticosus	-
		Plantago media	-	XIII.	1.	Papaver rhoeas	-
		Scabiosa columbaria	-			Tilia platyphyllos	-
V.	1.	Solanum dulcamara	-			Papaver orientale	-
		Atropa belladonna	-	2.	Paeonia peregrina	-	
		Nicotiana rustica	-			Aconitum napellus	-

Class	Order	Specification of Plants	Toxicity of Pollen	Class	Order	Specification of Plants	Toxicity of Pollen
XIII.	3.	Trollius europaeus	-	XIX.	2.	Arnica montana	-
		Ranunculus auricomus	-			Matricaria chamomilla	-
		Anemone pulsatilla	-			Achillea millefolium	-
		Anemone sylvestris	-			Artemisia absinthium	-
XIV.	2.	Digitalis purpurea	-			Artemisia vulgaris	-
XV.	2.	Arabis arenosa	-			Centaurea cyanus	-
		Brassica Napus	+		4.	Calendula officinalis	-
XVI.	2.	Geranium sylvaticum	-	XXI.	1.	Euphorbia Gerardiana	+
		Geranium pratense	-		3.	Zea Mays	+
	5.	Lavatera thuringiaca	-			Carex vulgaris	+
		Malva sylvestris	-			Carex intermedia	+
		Malva alcea	-			Carex arenaria	+
		Althaea rosea	-			Carex paniculata	+
		Althaea ficifolia	-			Carex glauca	+
XVII.	4.	Cytisus laburnum	-			Carex alba	+
XVIII.		Hypericum quadrangulum	-			Carex verna	+
XIX.	1.	Arctium lappa	-		4.	Urtica dioica	-
		Carduus acanthoides	+		5.	Ambrosia trifida	+
	2.	Leucanthemum vulgare	-			Xanthium macrocarpum	-
		Solidago odora	+			Iva xantifolia	-
		Solidago nemoralis	+	XXII.	2.	Salix	-
				XXIV.	1.	Lycopodium clavatum	-

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AN INVESTIGATION INTO THE CONDITIONS AFFECTING
THE OCCURRENCE OF TYPHOID FEVER IN BELFAST.

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FOR a considerable number of years, Belfast has suffered from a high typhoid fever death-rate, and the investigations embodied in the following pages were undertaken at the request of the Corporation, with the purpose of determining the sanitary measures required to prevent, as far as possible, the occurrence of typhoid fever in the city.

Belfast is situated on the banks of the river Lagan, as it falls into the Lough, and alluvial blue clay forms the foundation of the older quarters of the city. The level of this district is very little above that of high water. The more recently built quarters are situated on ground outside this area, where the foundation is composed of heavy red clay, which is very impervious to moisture. The level there gradually rises up to 150—200 feet above high water.

Since 1897, the typhoid problem has been the subject of repeated investigations, and the reports furnished to the Public Health Committee on various aspects of the question have afforded valuable material for the present enquiry.

There are no accurate records of the history of typhoid fever in Belfast until the year 1881. In that year, for the first time, death returns due to this disease were given in a separate group. The following table gives the annual death-rate, per 10,000 of the population, from typhoid fever, during the years 1881—1902.

Typhoid Fever in Belfast

Year	Annual death-rate			Year	Annual death-rate		
1881	3·7	1892	4·1
1882	2·9	1893	4·4
1883	2·8	1894	5·1
1884	2·4	1895	6·2
1885	2·2	1896	4·5
1886	3·9	1897	11·4
1887	3·5	1898	18·8
1888	3·3	1899	7·5
1889	7·9	1900	7·2
1890	7·6	1901	9·7
1891	5·9	1902	4·7

The fact brought out in this table which at once calls for attention is the rise in the year 1889. The table includes 22 years. During the eight years that preceded 1889, the average death return was 3·1 per 10,000 of the population. During the 14 years since that date, the average has been 7·5, and throughout this latter period, it has not once fallen to 3·1. In the course of these 22 years certain essential changes in the sanitary condition of Belfast have been effected. The city as it existed in 1870—1880 had many sanitary defects, which have since been removed, or minimised. At that date, there was no system of sewage disposal. The sewers opened into the Lagan and other available streams. Domestic sanitation was exceedingly defective. The drains of many of the houses were not constructed so as to prevent leakage of the sewage. They were often made of bricks or timber. The houses of the working classes were nearly all provided with privies and ash-pits. The method of cleansing the ash-pits was very defective. Many of these houses were built without back passages, so that when it became necessary to have the ash-pit cleansed the contents had to be carried through the house. To add still further to the sources of filth about these small houses, the practice of keeping pigs was a general one, even in houses which were without back passages. These insanitary conditions were gradually dealt with by the Public Health authorities. In 1888, the main Drainage Scheme was commenced, and this was completed in 1896. In 1889, the registration and regulation of dairies within the city boundaries were introduced. In 1890, the Public Health Officers began to test all drains constructed under their supervision, by the smoke and water test. In 1892, ash-pit cleansing was undertaken free of cost by the Corporation, and regulations regarding the proper construction of the ash-pits were introduced. Prior to this the structure of the ash-pit was very defective. The floor was not concreted, and was therefore pervious. The level was

below that of the surface of the yard, and consequently the liquid contents could not escape. Further, there was no roof, and the rain washed the contents into the soil. The ash-pit indeed became a small leaking cesspool. The remedying of these structural defects has greatly improved the surroundings of the houses, by diminishing the amount of surface filth. Prior to 1892, also, the Corporation had a rule according to which the householders were compelled to pay 1s. for each cart-load of refuse that was removed from the ash-pits. As a result of this rule, the cleansing of the ash-pits was very much neglected, and many of the houses became surrounded by accumulations of filthy refuse. Advantage was taken of any convenient space near, wherein to deposit the refuse that could not be accommodated in the yard. Back passages were often utilised for this purpose. There was, therefore, round the houses of the working classes, a great amount of dangerous surface pollution. The Superintendent of the Cleansing Department informed me that when the cleansing regulations of 1892 came into force, he removed from these houses an average of two loads per house. At present the average cleansing yields one load from every 10 houses. The number of privies has been steadily reduced; especially since 1899, when power was obtained to compel owners of houses to substitute water-closets for them. Since 1894, the drains of all new houses having internal water-closets have been subjected to the water test, and latterly (1898) this test has been applied to new house drains of all kinds. In 1894, regulations regarding the keeping of pigs were introduced, and the new rule that the pigsty must be 15 feet away from the dwelling-house resulted at once in the abolition of nine-tenths of the pig-keeping. In 1894, the Corporation established a disinfecting station. In 1897, the Infectious Diseases Notification Act was adopted.

It is beyond question, therefore, that since 1888 a great advance has been made towards cleanliness in the city. Since the Main Drainage Scheme was commenced, a sanitary reconstruction of the city has been in progress. The question may be asked 'how far the introduction of the regulations just described has succeeded in abolishing the sanitary defects towards the removal of which they were directed.' The data for answering this question have been already partly supplied in the description of the regulations for the cleansing of ash-pits, the regulations regarding pig-keeping, etc.

As regards dwelling-houses, two questions fall to be considered: (1) House accommodation, and (2) Domestic Sanitary arrangements.

The data regarding house accommodation are furnished by the census of 1901. The city has been increasing rapidly in size. In 1881, there were 208,122 inhabitants, and in 1901 there were 348,705. In spite of this rapid increase in the number of the inhabitants, the house accommodation provided in Belfast is better than that of any other county borough in Ireland. In place, however, of comparing Belfast with other towns in Ireland, it may be more instructive if we put side by side the census returns of Belfast, Liverpool, and Manchester.

House accommodation in Belfast, Liverpool, and Manchester, in 1901.

		Number of Rooms in Tenements	Tenements of less than 5 Rooms	Death-rate from Typhoid per 10,000 of Population	
BELFAST.					
Total tenements	69,981	1	697	1898—18·8	} average 9·5
Tenements of less than 5 rooms }	32,008	2	4,874	1899— 7·5	
		3	5,086	1900— 7·2	
		4	21,351	1901— 9·7	
				1902— 4·7	
LIVERPOOL.					
Total tenements	138,845	1	8,527	1898— 2·2	} average 2·2
Tenements of less than 5 rooms }	46,822	2	11,635	1899— 2·6	
		3	13,010	1900— 1·7	
		4	27,433	1901— 2·2	
				1902— 2·2	
MANCHESTER.					
Total tenements	112,854	1	2,140	1898— 2·2	} average 1·4
Tenements of less than 5 rooms }	61,572	2	6,869	1899— 1·2	
		3	4,994	1900— 1·2	
		4	47,569	1901— 1·3	
				1902— 1·1	

The house accommodation is very similar in Belfast, Manchester, and Liverpool. In Belfast 45 per cent., in Liverpool 34 per cent., and in Manchester 54 per cent. of the tenements have less than 5 rooms. Liverpool, however, had about $\frac{1}{4}$, and Manchester about $\frac{1}{6}$ of the Belfast mortality from typhoid fever in the last five years. It is clear therefore that it is not to defective house accommodation that we have to look for the cause of the excessive typhoid of Belfast.

Domestic sanitation. The data on this subject were obtained in a special investigation of five selected representative areas of the city. I propose to give these results in the tabular form in which they were furnished to me by the Sanitary Inspectors, and then to discuss the conclusions to which they lead.

General Description of the Selected Areas.

Area A—District¹ No. I.: This district, which covers an area of 10 a. 1 r. 12 p., is built entirely on blue clay, with high sub-soil water. Population, 3,400. The average elevation is 3 feet above high water. The leading streets are of good width (40 to 60 ft.), the smaller streets from 25 to 35 feet; and they are kept clean. The houses are very old, and are occupied chiefly by the artisan and labouring classes. Many of these houses have no back passages.

Area B—District No. III.: This district, which covers an area of 16 a. 3 r. 21 p., is built on the red clay, and has a low sub-soil water. The average elevation above high water is 99 feet. The streets average a width of 35 feet, and are clean, bright, and airy. The houses, which are occupied by the artisan class, are of modern type, and were built about 10 to 14 years ago. They are let at from 3s. to 5s. per week. The houses have back passages. Population, 4,439.

Area C—District No. IV.: This district covers an area of 14 a. 3 r. 20 p., is built entirely on made-up ground, filled in with road-scrappings, ash-pit and town refuse: sub-soil water high. The average elevation above high water is 10 feet. The streets are wide (35 and 40 feet), and none less than 30 feet. They are clean, bright, and airy. The houses, which are occupied by the artisan class, are of modern type, and with the exceptions of Boyne Square (built about thirty-eight years ago), Roland Street (very old portion), and part of Clementine Street (built about twenty-five years ago), were erected from ten to fourteen years ago. They are let at about 3s. 6d. per week. City Street is a better class property, and is let at 4s. 6d. to 5s. per week. Population, 4,336. The majority of these houses have back passages.

Area D—District No. VI.: Average elevation, 10 feet. This district, which covers an area of 12 a. 2 r. 3 p., is built the higher portion (next Divis Street) on the red clay, the lower portion (next College Square North) being built on the blue clay, with high sub-soil water. The streets are narrow, being about 25 feet wide, and are hard to keep clean—the residents frequently throwing their slop water on to them. The houses, with the exception of those abutting on College Square North, Durham Street, Divis Street, and King Street, are those of the lower labouring class, and let at about 2s. 6d. per week. With few exceptions these houses have no back passages. This district is being gradually improved. Population, 2,205.

Area E—District No. X.: This district, which covers an area of 65 a. 1 r. 23 p., is built on the red clay and has a low sub-soil water. The average elevation of the district above high water is 160 feet. The streets are wide, having an average width of 35 feet. They are clean, bright, and airy. The houses are chiefly occupied by the artisan class, are of modern type, and were built about eight years ago. They are let at from 3s. 6d. to 5s. per week. These streets have back passages. Population, 11,270.

A thorough examination of the houses in these areas has been

¹ 'District' refers to Dispensary District, according to which the typhoid returns are classified.

carried out by the Sanitary Officers in charge of the districts. The results are given in the following table:

Experimental Area	Dispensary District in which situate	Total Number of Houses	Percentage of Houses with Water Closets	Percentage of Houses with Privies	Percentage of Houses with Covered Ash-pits	Percentage of Houses with Uncovered Ash-pits	Percentage of Houses with back passage accommodation	Percentage of Houses without back passage accommodation	Remarks
A	1	694	82 %	18 %	56 %	*38 %	50 %	50 %	*6 % have ash boxes
B	3	906	91 %	8 %	67 %	33 %	99 %	1 %	
C	4	885	93 %	7 %	57 %	*41 %	62.65 %	37.35 %	*2 % have ash boxes
D	6	450	39 %	61 %	26 %	74 %	18 %	82 %	
E	10	2,300	87 %	13 %	96 %	4 %	86 %	14 %	

In the next place the drains were all examined by the smoke-test. The results were somewhat incomplete, but indicated that a proportion of the drains were defective:—

Area A. On applying the smoke-test 33 per cent. of the drains were found defective, but the remainder cannot be guaranteed perfect, as the test may not have been effective owing to the smoke probably escaping into the sewer.

Area B. On applying the smoke-test 73 per cent. of the drains were found defective.

Area C. On applying the smoke-test 32 per cent. of the drains were found defective, but the remainder cannot be guaranteed perfect, as the test may not have been effective in some cases owing to the smoke probably escaping into the sewers.

Area D. On applying the smoke-test 47 per cent. of the drains were found defective, but the remainder cannot be guaranteed perfect, as the test may not have been effective owing to the smoke probably escaping into the sewers.

Area E. On applying the smoke-test 37 per cent. of the drains were found defective.

The sewers were also examined by members of the City Surveyor's Staff. According to the observations, the sewers of each street were classified as in 'good,' 'fair,' or 'bad' state of repair.

I give in tabular form a summary of the elaborate observations which were made.

Area	District	Number of Sewers examined	State of repair of Sewers			Percentage of good
			Good	Fair	Bad	
A	I	27	12	12	3	44
B	III	14	14	0	0	100
C	IV	17	14	0	3	82
D	VI	26	20	0	6	77
E	X	34	34	0	0	100

The following table gives the number of cases of typhoid fever notified, per 10,000 of the population, in each of the five experimental areas during the 3 years 1900—1902:

		1900	1901	1902	Dispensary District
Experimental Area,	A	126·4	64·7	32·3	No. 1
„	„ B	126·1	153·1	45·0	No. 3
„	„ C	59·9	57·6	29·9	No. 4
„	„ D	54·4	99·7	18·1	No. 6
„	„ E	60·3	139·3	26·6	No. 10

The foregoing table will be more easily understood when it is compared with the next, which gives, in a similar way, the number of cases of typhoid fever, per 10,000, in each Dispensary District, for the same period.

Table showing by Registrar's Districts the number of cases of typhoid fever notified, per 10,000 of the population.

		1900	1901	1902
City		50·9	72·4	29·0
Registrar's District No.	1	56·4	55·1	31·3
„	„ 2	43·7	63·3	28·3
„	„ 3	79·1	99·5	34·7
„	„ 4	42·9	54·8	19·7
„	„ 5	44·7	92·4	34·1
„	„ 6	34·7	46·8	17·9
„	„ 7	—	—	—
„	„ 8	28·0	66·0	32·0
„	„ 9	48·2	56·4	21·0
„	„ 10	104·7	164·3	43·0
„	„ 11	35·6	42·8	33·6
„	„ 12	48·0	94·3	40·0
„	„ 13	34·0	46·0	37·3
„	„ 14	—	—	—

Typhoid Fever in Belfast

The following table shows in the same Districts the annual death-rate per 10,000 of the population from typhoid fever during the years 1900, 1901, and 1902.

District	1900	1901	1902
City	7·2	9·7	4·7
No. 1	8·1	8·8	7·4
2	8·7	8·9	2·7
3	10·0	12·5	4·2
4	7·0	9·5	7·3
5	7·0	12·9	7·0
6	5·4	6·4	2·7
7	—	—	—
8	12·0	2·0	8·0
9	4·1	5·6	4·1
10	14·3	18·2	4·8
11	4·5	8·3	5·7
12	6·2	12·8	5·0
13	4·6	3·3	6·7
14	—	—	—

Unfortunately, the present arrangement of the Dispensary Districts of the city has been in force only since 1900. Hence it is impossible to compare the returns given in the foregoing tables with those obtained in earlier years.

Of the three years included in the table, 1900 and especially 1901 are years in which typhoid fever had assumed the epidemic form. In 1902 it became quiescent, and in this condition it has since remained. It is important to notice that the increase of cases notified in 1901 as compared with 1900 is to be observed in practically all the districts of the city.

District No. 10 which, during the years of the epidemic, gave by far the largest return of cases, has had the same pre-eminence since 1897, when typhoid fever became so firmly established in the city.

Sites.

In the light of these results, we may, in the first place, take up the discussion of the problems connected with the *sites* of the houses.

The chief evil that exists in relation to the sites is that of making the foundations of houses in a layer of recently deposited city refuse. The city refuse, derived as it was from the privies and ash-pits to a considerable extent, contained a large admixture of organic pollution, part of which must undoubtedly have been infected with typhoid bacilli.

This method of making foundations was most extensively adopted in constructing the streets which now occupy the ground formerly known as Crawford's Brickfields, in No. 4 district. These streets were built in the years 1880—1895. Such ground should not be used as a site for houses till the natural process of purification in the soil has occurred. This precaution was not taken, however. An examination of a group of houses in that district has been carried out by the sanitary officers, and the results are included in the foregoing tables—Experimental Area C, District No. 4.

The houses in this experimental area are fairly well provided with water-closets (93 %). Therefore, while a considerable number have uncovered ash-pits (41 %), and further, 40 % have no back passage; yet the evil of these defects is much lessened by the relative absence of privies. This property is sufficiently good in other respects to permit us to regard the district in which it is situated as a fair test of the evil which arises from building on sites of the imperfect character described. District No. 4 is the part of the city where the largest amount of building on "made soil" has occurred; yet it does not occupy the position of a centre of typhoid infection.

The following is the record of the 3 years 1900—1902:

	1900	1901	1902
Cases notified per 10,000 of the population of the City	50·9	72·4	29·4
District No. 4	42·9	54·8	19·7
Experimental Area C in/ District No. 4	59·9	57·6	29·9

The district as a whole is distinctly below the average for the city and the experimental area is during 1901 distinctly below, and in 1902 practically the same as, the average. If the originally contaminated condition of the soil, and the soaking of sewage into the sub-soil in this area, were a primary source of typhoid infection, we would have had clear evidence of it in these returns.

Further, in an investigation on the infection of the soil, I have found that the samples of "made soil" from the district just discussed had no special power of sustaining the life of the typhoid bacillus. The bacillus in such soil would rarely survive for more than a few days.

It should be explained at this point that the danger due to contamination of the soil in Belfast has fortunately not been complicated by the use of the sub-soil water for drinking purposes. In former years there were a few wells; but they were never used in large

numbers, and now they are all closed. On this account the conditions in which soil contamination can become effective are clearly defined. Faecal matter, mixed with a large quantity of soil, is relatively harmless; but the sewage which escapes into the soil from the drains, if it reach the surface, becomes a cause of infection.

As regards the relation of conditions of the soil to the occurrence of typhoid fever, there is further evidence in the fact that in Area E, from District No. 10, we have an example of a virgin soil of red clay as the foundation of the houses; yet the typhoid returns show that this is one of the worst parts of the city.

There is, therefore, no evidence in these returns to show that the character of the soil used as the foundation of the houses has an influence of primary importance on the spread of typhoid fever.

Sanitation of Houses.

The next point which is clear from these tables, is that the Area D, in District 6, is by far the worst of the selected areas as regards the sanitary state of the houses. Only 39 % of the houses have water-closets, only 26 % have covered ash-pits, and only 18 % have back passages. Further, a considerable number (47 %) of the drains were ascertained to be defective. The condition of the sewers is also one of the worst among those examined (23 % defective). The streets are narrow (25 feet wide), and they are difficult to keep clean owing to the careless habits of the occupiers of the houses. In an area containing houses of this insanitary description, as we might well expect, typhoid was plentiful (99·7 cases per 10,000 of the population) in 1901, when it was prevalent in the whole city. It was, however, much more plentiful in the Areas B (153 per 10,000), and E (139 per 10,000), taken from Districts 3 and 10 respectively.

Areas B and E are in many respects similar in character. Both are high lying. The houses are built on the red clay. The sub-soil water is low. The streets are 35 ft. wide, and are clean, bright, and airy in each case. The houses are modern in structure, and they are occupied by the same class of people. The sewers are, without exception, in a good state of repair in each case. In Area E 37 % of the drains were defective, and in Area B 73 % were similarly out of order. The most important defect which was discovered in B was the state of the drains. I made enquiry as to the reason why this area, with houses built within the last 10 years, should compare so

unfavourably with others in which the houses are much older. The explanation of the leakage from the drains is the fact that the joints of the drain pipes were made with clay. Clay is too readily washed away to form a suitable joint. It is difficult, however, to estimate the relative importance of this defect. In Area E, which had about the average number of defective drains, or about half the number that were found defective in B, the prevalence of typhoid fever is nearly the same as that in B, and both show much higher returns than any of the other areas. The evidence relating to drain defects is incomplete as regards the other three areas. This fact is due to a faulty condition of the drains, generally the absence of inspection chambers. In B and E, however, the test was satisfactorily applied, and the results are sufficient to show that the defects of the drains in B did not greatly add to the liability to typhoid fever of B as compared with E. On the other hand, when we compare E with D, as regards sanitary conditions generally, we observe that E stands far ahead in every respect, and yet it had a much higher return of typhoid cases in the epidemic of 1901.

In order to obtain further evidence on the relation of defective drains to the occurrence of typhoid fever, I asked the sanitary inspectors to furnish me with the results of their experience in this matter. The following table gives the results during three representative periods:

	Total Number of Houses where drains were tested	Houses with defective drains	Percentage of defective drains in the total number	Number of Houses in which Typhoid Fever had occurred	Number of Typhoid Houses with defective drains	Percentage of Typhoid Houses with defective drains
			Per cent.			Per cent.
Northern Division, 1st Feb.—30th March, 1901 }	85	52	61·2	64	33	51·56
Southern Division, 1st Feb.—30th March, 1901 }	65	34	52·3	30	12	40
Northern Division, 1st July—31st Dec., 1901 }	715	363	50·76	625	292	46·72
Southern Division, 1st July—31st Dec., 1901 }	764	446	58·3	533	309	59·3
Northern Division, 6th Nov., 1902—15th June, 1903 }	245	138	56·32	142	70	49·29
Southern Division, 6th Nov., 1902—15th June, 1903 }	248	119	48	137	58	42·4

These returns afford clear evidence that the houses in which typhoid

cases occur are not specially characterised by the possession of defective drains.

As regards Area A, the results of the investigation are interesting, chiefly in relation to the question of elevation. This it is to be noted is only 3 feet above high water. The houses are old, the foundation is the alluvial blue clay, and the sub-soil water is high. The sewers are distinctly bad. Yet in the epidemic of 1901 the number of typhoid cases was decidedly below the average of the whole city.

The investigation has revealed the presence of sanitary defects in the areas examined, and these areas have been selected as typical of the various districts of the city. These defects all more or less contribute to the conditions which make it easy for typhoid fever to find a footing. There is no evidence, however, from the investigation to show that the peculiar burden of typhoid fever which is laid upon the city is due in the first instance to these defects.

Infection of the Soil.

A further effort was made to define the extent to which defective sanitation in connection with the houses might be regarded as the means of spreading typhoid infection. From leaking house drains, etc., the soil is being more or less constantly inoculated with the typhoid virus. It becomes important therefore to determine how far the soil may be regarded as a medium in which the *Bacillus typhosus* can maintain its life. This problem is a most complicated one, since we are to a large extent without a knowledge of the physical and chemical conditions of soil which are favourable or otherwise to bacterial life. A certain amount of research bearing on the particular question before us has been carried out. It has, however, been limited chiefly to the question of how long the typhoid bacillus will survive in various samples of soil. At the request of the City Council I carried out an investigation upon this subject. The results, the details of which I need not at this time enlarge upon, go to support the conclusion that, as a rule, the typhoid bacillus is unable to survive for more than a short period in natural soils, or even in soils which have been sterilised before the typhoid bacillus has been planted in them.

An examination of the bacteria present in soils which are subject to contamination, showed that not only typhoid bacilli, but also *Bacillus coli communis* are absent, unless the contamination has been very recent.

On the other hand the durable spores of *Bacillus enteritidis sporogenes* were found widely distributed in such soils.

We cannot, therefore, regard the soil as an enduring source of typhoid infection simply because it has at some time been inoculated with the specific bacillus. The soil acts as a filter, and the sewage which passes through any considerable layer of it loses the typhoid bacillus which it may have originally carried. When the proportion of sewage is very large in relation to the soil in which it is contained, or when it escapes on the surface of the soil, we have to deal with conditions which are, bacteriologically speaking, essentially different from those presented in the problem of infected or inoculated soil. The conditions which arise in these circumstances form part of the general question of surface pollution.

Infective material which finds its way to the surface of the soil by the escape of sewage, or otherwise, is regarded by all investigators as one of the most fertile sources of typhoid disease. Such material may be conveyed to susceptible individuals by various means. It may be carried into the house, as Koch has pointed out, on the feet or hands of children. It may be blown in the form of dust by the wind. It may adhere to the feet of flies and be taken directly to the food. It may be washed into streams and wells from which drinking water is obtained. In these cases the question of the survival of the typhoid bacillus takes a secondary place, while the mode of conveyance of the virus from the surface of the soil to the new patient becomes the primary consideration. In order, however, fully to appreciate the danger which arises from this source of infection, survival of the bacillus must be studied experimentally here also. The problem arises in the investigation of many forms of typhoid epidemic. One illustration of its importance may be quoted. The explanation given of the cause of the typhoid epidemic in Paris in 1894, is that a heavy fall of rain had washed a large amount of contaminated surface matter into the Vanne, from which a section of Paris obtains its water supply. The point of special interest is that although a typhoid epidemic had occurred recently in the Vanne area, a year had elapsed since there had been any cases. The most obvious interpretation of this sequence of events is that the typhoid virus persisted in an effective condition for a year in the surface layers of the soil. That this may have taken place, however, requires further demonstration, especially since other explanations might be offered.

Direct Infection.

Attention has been directed anew to surface pollution by Koch in his recent enquiry into the continuance of endemic typhoid fever in the villages at Trier¹. Koch, however, regards the direct transmission of the virus from one individual to another by means of surface pollution as a more important consideration than the survival of it in the surface layers of the soil. According to this hypothesis it is of primary importance to isolate the infected members of the community, and destroy the bacteria in the dejecta, in order to prevent the spread of the disease from one individual to another. Hence, he advocates the necessity for hospital treatment of all cases, and the isolation of those who, though they are not, medically speaking, suffering from typhoid fever, are infected in the sense that an examination of the faeces shows that the typhoid bacillus is present in the intestine.

How far the same explanation would hold for a large city in which, admittedly, surface pollution is abundantly present, has not been determined. Certain data are already in our possession, however, which throw light on the distribution of cases in Belfast. The record of infectious diseases notified to the Medical Officer of Health is so kept that it is possible to classify the houses in which typhoid or other infectious diseases occur, according to the number of cases in them. In the following table I have given the results of a classification of the houses and cases on this basis:

Year	Total number of cases	No. of Houses in which Typhoid cases occurred	Percentage of Houses with one case	Percentage of Houses with two or more cases	Average No. of cases per House, where more than one occurred	Percentage of the cases possibly accounted for as direct infection from the first case in the House	Percentage of the streets in which Typhoid fever occurred, in which there was one case only
1898	5136	4301	86·4	13·6	2·4	16·2	
1899	1598	1403	88·4	11·6	2·2	12·2	
1900	1777	1514	87·6	12·4	2·4	14·9	50·0
1901	2503	2099	86·3	13·7	2·4	16·1	39·3
1902	1044	925	89·2	10·8	2·2	11·4	60·0
1903	842	775	92·9	7·1	2·2	7·9	

From this table it follows, that in fully 85 % of the houses in which typhoid fever occurred there was no second case. It is difficult to

¹ Koch, *Die Bekämpfung des Typhus*, 1903.

reconcile these results with the hypothesis that the primary element of the typhoid problem in the city of Belfast is direct infection. If within the circle of closest personal contact, 70 % of the cases show no evidence of the infection being conveyed from them to others by direct contagion, there seems little ground for trying to link together a chain of cases to explain the spread of infection by direct means outside the household.

I have added the returns per street for three years, and it is found that about 50 per cent. of the streets in which typhoid broke out, have only one case. There is, therefore, nothing in the record of even this wider circle to lead us to believe that direct infection gives rise to more than a minority of the cases.

The conclusions from these tables are subject to various fallacies. In the first place, the table includes cases of simultaneous infection from a common source. Such cases do occur, and if we had the means of separating them from the others, their exclusion would still further lower the number of cases which could be ascribed to direct infection. In the next place, there is no doubt that a large number of typhoid fever cases occur in which the patient never comes under medical treatment. The symptoms are trivial, or they may be in a large measure absent. Such cases, or a large part of them, may, for reasons to be discussed, be regarded as forming examples of that indefinable clinical entity to which the name of "continued fever" has been attached. I have worked out the classification of cases of "continued fever" according to house distribution in 1901. In that year there were 1351 cases of "continued fever" notified. These occurred in 1212 houses, and in 99, or 8.1 %, of these houses there were more than one case. Hence, if we include the whole of the cases of "continued fever" amongst those of typhoid fever, the percentage of houses in which one case occurred would be relatively increased. The classification by houses of other infectious diseases notified to the Medical Officer of Health should be compared with that of typhoid fever. The numbers of these are however so much smaller than that of the typhoid fever cases that the comparison is probably fallacious. The cases of diphtheria in 1900 numbered 321, and these occurred in 265 houses. In 230 houses there was one case, and in the remaining 35 houses (13.2 %) there were two or more cases. In 1900 there were 658 cases of scarlet fever. The number of houses invaded was 604, and of these 99, or 19.6 %, contained more than one case.

The result is then as follows:

	Percentage of Houses with more than 1 case
Typhoid Fever epidemic	13·6 %
Typhoid Fever moderately quiescent	7·1 „
Continued Fever abundant	8·1 „
Scarlet Fever moderate	19·9 „
Diphtheria quiescent	13·2 „

These results are modified to a certain extent by the isolation and treatment of cases in the Fever Hospital. They are of importance from the practical point of view, because of their bearing on that question. In Belfast the treatment of cases of typhoid fever in hospital has not been extensively carried out. Only about 50 % of the cases notified are so treated. It should, however, be pointed out that this estimate of 50 % is probably a very misleading one. As we have suggested, many cases notified as cases of "continued fever" are in all probability cases of typhoid. One of the most valuable portions of Koch's recent treatise is that in which he deals with this subject. He was able to demonstrate, by the Conradi and Drigalski method, the existence of infection in cases which, clinically speaking, would not be notified as "typhoid." That these cases are plentiful has long been suspected, and that many of them find their way into the group of cases of "continued fever" seems certain, if we compare the returns of typhoid fever and "continued fever" year by year.

	1897	1898	1899	1900	1901	1902
Cases of Typhoid Fever	3269	5136	1598	1777	2530	1044
Cases of simple Continued Fever	378	1321	691	813	1351	730

There is a sufficient correspondence in the rise and fall of the numbers of cases of the two diseases to indicate a very close relation between them. If we further computed the numbers by a calculation on the basis of Koch's results at Trier, it would become clear that a relatively small percentage of the cases capable of causing infection find their way into hospital. The hospital treatment which does exist in Belfast, therefore, is not extensive enough to be a source of serious fallacy in the interpretation of this table of the distribution of cases.

We are therefore led to the conclusion that direct conveyance of the virus from one individual to another does not hold the same position as a means of spreading infection in Belfast, as that ascribed to it by Koch in the endemic typhoid at Trier.

Before leaving this aspect of the subject, reference should be made to the less obvious avenues of infection, some of which, in other places, and in altered conditions, are of much greater importance than they appear to be in Belfast.

Dust.

Admitting as we do that pollution of the surface of the soil is one of the more important sources of typhoid infection, it would seem highly probable that the infective material, when it has dried, may, in the form of dust, be conveyed by currents of air into the houses, and be deposited in the water, or milk, or other nutrient fluids, in which the bacillus is able to grow. Belfast is not specially subject to the dust which is blown from the surface of the soil by the wind. The rain-fall, while not excessively high, is distributed over a large number of days in the year. Hence dusty weather is a comparatively unusual experience. Further, there is no evidence in the record of streets invaded by typhoid to support the hypothesis of the conveyance of infection by dust. The streets which suffer chiefly from dust are those in which there is a large stream of traffic between the city and the surrounding country. The houses in these streets are not specially liable to typhoid.

Flies.

Similarly, the part taken by flies in the spread of typhoid fever is probably a relatively small one in the climate of Belfast. In warmer climates, such as that of South Africa, or Egypt, where flies are extremely plentiful, the dissemination of typhoid bacilli may in some cases be due to their agency. The hypothesis involves conditions the existence of which in our climate has not been demonstrated.

We may here appropriately discuss the conveyance of typhoid infection through two articles of food—shell-fish and milk.

Shell-fish.

The dissemination of typhoid bacilli by shell-fish has special importance in Belfast, from the fact that at present the mode of disposing of the sewage of the city is to discharge it into the ebb-tide, at a point in the Lough about a mile below the mouth of the harbour. The result is that the tidal water at the head of the Lough contains about 4% of raw sewage. On either side of the central channel in the Lough there

is a large area of land, which is covered with water at high tide, and exposed at ebb-tide. From this area, which is known as the "slob land," large quantities of shell-fish are obtained. The returns of the Fisheries Department show the extent to which the industry of gathering shell-fish is pursued. The annual average amount of shell-fish obtained from the Lough during the last seven years has been the following: Periwinkles, 31 tons; Mussels, 413 tons; Cockles, 9200 gallons; Oysters, 241 hundreds; Lobsters, 5190; Crabs, 5000. A large part of the shell-fish is exported from Belfast to be used as bait. On the other hand, there is no doubt that a certain part of it is obtained by the hawkers to be sold in the streets for food. Further, a number of people gather shell-fish on this land for their own use. There is no restriction placed on the gathering of the shell-fish, and people may be frequently seen busy at this work on the area immediately round the place where the sewage is discharged.

In 1902, I made, at the request of the Corporation, an investigation into the kinds of bacteria to be found in shell-fish gathered from the "slob land." The examination of the fluid obtained from the interior of the shell showed the presence of streptococci, amounting in some cases to 1000 per c.c., and of *B. enteritidis sporogenes*, amounting to more than 10 per c.c.; while the cultivation of the bacteria adherent to the gills in phenolated broth, showed the presence of *B. coli communis*, and coli-like forms. Further, 1 c.c. of the fluid injected into a guinea-pig subcutaneously caused death in 48 hours, and from the oedematous fluid which developed, a pure cultivation of typical *B. coli* was obtained. As a control investigation, I examined a second series of cockles procured from an area free from the suspicion of sewage. Eight cockles were examined: but in none of them were any of the three sewage bacteria found to be present.

Cases of typhoid fever have been reported to the sanitary authorities of Belfast, where, upon investigation, it seemed clear that the source of the infection was in all probability the shell-fish from the Lough, which the patients had eaten. The relatively small number of these cases, however, indicates that infection from this source is not more than a subsidiary cause of the spread of typhoid. For many years there has been a constant stream of typhoid-infested sewage discharging into the waters of the Lough, and infection from the shell-fish in these conditions is doubtless continuous, but it is limited in its amount by the fact that the habit of consuming shell-fish bought in the street is confined to a small section of the community.

Milk.

The other article of food which we must consider is milk. The milk is brought into the city of Belfast from a large number of small dairy farms. The great majority of these are situated outside the municipal boundary, and are therefore not subject to the regulations imposed on dairies by the city council. In a certain number of cases there has been the strongest ground for suspecting that typhoid infection had been conveyed through the milk.

Outside the municipal boundary the Notification of Infectious Diseases Act is not in force. Hence, the occurrence of typhoid in any such district, is unknown to the authorities. It is therefore difficult to make a complete investigation of suspicious cases.

An enquiry regarding the cause of the epidemic in 1901 was carried out by Drs Gardner Robb, Fulton, and Whitaker. They confined their enquiry to the examination of 24 houses in which typhoid fever had occurred, and their conclusion on the subject of milk as the source of infection is the following: "In two houses, we found that none but condensed milk was used. In 4, there was no regular source of milk supply. The remaining 18 drew their milk from 12 different sources. Enquiry at these places failed to elicit any evidence of the possibility of infection by that means."

The outbreaks of typhoid fever which have been justly ascribed to milk infection have been limited to small groups of patients, and since the milk industry in Belfast is in the hands of such a large number of dairymen, it is improbable that a widespread epidemic could arise from this form of infection.

Water.

We have still, in our study of the conditions affecting the occurrence of typhoid fever in Belfast, to consider the subject of the water supply; and the fact that cases of typhoid fever have taken place in the catchment area at once invests it with importance in the discussion.

The water supply of the city is under the direct control of a Corporate Body, established in 1840, and known as the Belfast Water Commissioners. The water is obtained from three sources: the Woodburn, Stoneyford, and Mourne supplies.

The Mourne supply is being gradually introduced at the present time. The water is of the highest quality; but it has not as yet

been in use to any extent. The health of the city can, therefore, be considered only in relation to the two older sources of supply.

The supply from the Woodburn district was first obtained in 1865; but the scheme was not completed until 1879, or thereabouts. The catchment area is situated 12—15 miles to the North East of the city, in the hilly land behind the town of Carrickfergus. The water collected there is brought to the filtering works in the neighbourhood of the city at Old Park, and from a service reservoir there it is supplied to parts of the city which are at a level of not more than 100 feet above Ordnance datum. This supply has been regarded with suspicion by the Commissioners. They pointed out that in the catchment area there is a considerable number of inhabitants among whom typhoid cases have occurred, and also that top dressing of the land with town manure from Carrickfergus is carried out. The area has deteriorated by the extension of farming of this type since the water was first taken from it in 1866. In 1893 the Commissioners obtained powers to enable them to control the pollution of the streams feeding the reservoirs; but the provisions of the Act were rendered nugatory by a clause which prevented them from interfering with tillage. In consequence of the futility of this Act further steps were taken in 1899, when powers were obtained to purchase large portions of the catchment area, and since then the Commissioners have been making use of the powers granted to them at that date.

The Stoneyford area is situated about 10 miles to the South West of Belfast, on the high lying ground near Lough Neagh, about 5 miles to the North of the town of Lisburn. The scheme for obtaining water from this area was adopted about 1884; but the works were not completed till August 1888. The water from this source was gradually introduced, and by April 1890 it was in general use throughout the city. The water from Stoneyford has been the only supply available for areas which are over 100 feet above Ordnance datum. The water is collected into large reservoirs, and is filtered at Stoneyford, before it is sent to the service reservoir at Lagmore.

The Stoneyford catchment area consists of 5348 acres, 60 % of which is pasture, and 40 % cultivated land. Situated on this area there are 114 dwelling-houses, with a total population of 544. From a sanitary point of view the district is far from satisfactory, and the Commissioners have made a number of attempts to deal with the difficulties arising from the imperfect sanitation of the catchment area. Early in 1903, as the result of an interview which they had with the

Local Government Board, Dr Clibborn, Local Government Inspector, was directed by the Board to report on the alleged pollution of this source of the water supply. The Public Health Committee of the Corporation put at his disposal the services of two highly trained sanitary sub-officers, and the following table contains the summary of their report:

APRIL, 1903.

SANITARY SURVEY OF CATCHMENT AREA OF THE
BELFAST WATER SUPPLY, STONEYFORD.

ABSTRACT OF REPORT.

Number of Dwelling houses	114
„ Schools	2
							Total	116
„ Cowsheds	98
„ Houses which have privy accommodation	14

This includes the two schools. These privies, with two exceptions, are improperly constructed, not being bottomed, thus allowing the liquid matter to percolate into the soil. There are no ash-pits at any of these houses. The drainage of the dwelling houses, cowsheds, and liquid matter from manure heaps discharges over the surface of the adjoining ground or field. At one of these houses the drainage discharges direct into the adjoining stream.

Number of Houses which have no privy accommodation of any kind, and where the slops of the dwelling houses and excreta are deposited anywhere around the house	102
Number of Houses where there is no apparent direct pollution of the adjoining watercourses	86

The drainage of these dwelling houses and cowsheds and liquid matter from manure heaps discharges over the surface of the grounds or fields adjoining.

Number of Houses where the drainage of the dwelling houses, cowsheds, and liquid matter of manure heaps discharges into the adjoining watercourses	29
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Ballymacward National School. The privy of this school is situated over a small watercourse that discharges into a stream which passes into Stoneyford reservoir.

Number of Houses with liquid manure tank	2
Number of Houses overcrowded	6
Number of Houses, on inspection, which were in a filthy state inside	7

In the great majority of the houses in the catchment area the surroundings were in a filthy state.

(Signed) HENRY REYNOLDS.
WILLIAM J. McBRIDE.

Typhoid Fever in Belfast

From this report it is clear that there is an almost total lack of ordinary sanitary arrangements in connection with the houses in the Stoneyford catchment area.

Further, on enquiring into the sanitary history of the district as regards typhoid fever, we find a record which corresponds with the defects in general sanitation.

The following table gives the return of typhoid cases in the Stoneyford area since 1897 :

*Return of Houses in Stoneyford Catchment Area in which
Typhoid Fever has occurred.*

Name	Date	No. of cases
P. ...	January, 1897	4
M'G. ...	August, 1899	1
Y. ...	July, 1899	1
M'C. ...	March, 1900	1
L. ...	February, 1901	1
M'M. ...	"	1
M'K. ...	October, 1902	1
" ...	March, 1903	1
D. ...	"	7
T. ...	April, 1903	1
H. ...	"	2
10 houses	6 years	21 cases

I have not been able to obtain the record of typhoid fever cases for the whole period since water from this supply was used in the city.

The table just given, however, includes the period during which the largest epidemics in the city have occurred, and during this time cases of typhoid fever have taken place in the small catchment area of 5000 acres, each year, except 1898. The relation of the time of occurrence of the cases in Stoneyford to the date of the commencement of the two large epidemics affords important evidence of the direct connection between these cases and the outbreak in Belfast.

The largest epidemic in Belfast, since 1881, occurred in 1897—98. It commenced in the city in March 1897. In January 1897, four cases occurred at P.'s farm in the Stoneyford catchment area.

Another epidemic occurred in 1901. This commenced in the city in the end of March. Two cases of typhoid fever occurred in different houses in the catchment area in February. In both these instances, there is good reason to connect the occurrence of the cases in Stoneyford with the epidemic outbreak in the city.

To this argument it may be replied, that the cases in 1902 and 1903 have not been followed by typhoid epidemics in the city. We have, however, somewhat different conditions. The filter beds at Stoneyford have been recently increased from four to six, and this makes an enormous difference in the efficiency of the filtration as a means of protecting the consumers. In the next place, the Commissioners have purchased about half of the cultivated land in the catchment area, and in this is included that portion lying nearest the reservoirs, and that which was regarded as the gravest source of pollution. These circumstances, coupled with the fact that the Commissioners have made renewed efforts to deal with each case as it arises, may be justly expected to yield beneficial results. A still further consideration of the greatest importance lies in the fact that typhoid fever has passed into a quiescent phase not only in Belfast, but in the surrounding towns.

A study of the incidence of typhoid fever in the different districts of the city brings out additional evidence regarding the direct effect of the Stoneyford water.

The complete details of the distribution of the water in the city are not easily made out, as there are areas where water from the two sources may be used. Since, however, the water from Stoneyford alone can be supplied to the parts of the city at a level of over 100 feet above Ordnance datum, we can in a measure trace the effect of this water on the typhoid fever rate. District No. X is the clearest example of a district supplied with undiluted Stoneyford water.

This district was the area of the most violent outbreak in the epidemic of 1897—98. Unfortunately, there has been a rearrangement of districts since that date, so that the exact returns are not available for comparison during the whole period since 1897. The returns for 1900—02 are available, and enable us to judge which district bore the brunt of the epidemic of 1901.

The following table answers the question :

No. of Cases of Typhoid Fever notified per 10,000 of the Population.

	1900	1901	1902
City	50·9	72·4	29·0
District No. X	104·7	164·3	43·0

Reference to the complete table, already given, will show that this district is by far the most typhoid-stricken area in the city.

The filtered water from the Stoneyford supply has been examined, at the request of the Water Commissioners, by Professor Frankland,

and his conclusion, in 1899, was the guarded one that it was of good quality "as far as it is possible to render water with this somewhat suspicious history satisfactory."

In 1898, I had the opportunity of making a bacteriological investigation of the water. The investigation was undertaken at the request of the Public Health Committee. I restricted myself practically to the question of determining whether I could detect in the water supplied to the city the typhoid bacillus, or the *Bacillus coli communis*. As regards the typhoid bacillus, the results were negative; but the *Bacillus coli communis* was present in considerable numbers. Some species were of virulent character. The importance of these observations, as far as they go, lies in their agreement with the general facts of the situation. In circumstances of a highly suspicious character, the filtration, and other precautions, were not sufficient to prevent the passage into the water to be supplied to the consumers, of bacteria of intestinal derivation. This bacteriological evidence proved the existence of a certain degree of pollution, and therefore of potential mischief, but did not prove specific pollution. Systematic bacteriological observation of the Stoneyford water has never been carried out, and hence, the case from this point of view, and independently of other facts, has never been completely investigated.

The evidence which I have summarised shows that the citizens of Belfast have been provided with surface and sub-soil water from a typhoid-infected area. The sanitary reform in the city has led to the closing of surface wells situated inside the municipal boundaries, in order to avoid the risk of typhoid infection from the water of the contaminated sub-soil. The contaminated sub-soil water in Stoneyford gives rise to the same risk; but the corresponding sanitary reform has not been carried out.

To protect the consumers however from the danger the Water Commissioners have adopted various expedients. In the first place, they have filtered the water through sand filters, and these filters have been recently increased from four to six in number. The construction of the filters and the rules applied in their use are similar to those adopted by the London Water Companies. The old established doctrine that filtration of water through sand filters is a relative, and not an absolute, safeguard has never been departed from. The security of the consumer, even apart from actual breakdown in the filtering arrangements, is a relative one, and the facts taken as a whole indicate that the security is proportional to the efficiency of the

filtration, on the one hand, and to the infective power of the virus, on the other. When the virus is powerful in virtue of its quantity or quality it will be conveyed through channels which are closed to a virus of less active character. Again, it cannot be inferred from the success of filtration in the treatment of Thames water by the London Companies that a similar degree of security will be gained from the application of the same process in circumstances such as those of Stoneyford.

The Water Commissioners have recognised that in times of danger extra precautions are necessary, and they have adopted the rule of turning off a stream, and allowing the water to run to waste, as soon as they know that a case of typhoid fever has occurred in any house on its banks. The application of this rule, however, is involved in very great difficulty. The Water Commissioners are not the Sanitary Authority of the area. Their attempt to obtain this power from Parliament was unsuccessful. The rural Sanitary Authority under whose charge the area remains has not adopted the Notification of Infectious Diseases Act. Hence, for lack of the necessary information regarding cases of typhoid fever, the Commissioners are unable to apply their rule with the promptitude required. It is also to be noted that Koch has shown recently that, along with cases of typhoid fever acute enough to require medical treatment, there is a large amount of latent typhoid fever. Hence, even the most complete system of notification would afford a protection of an insecure description. Nor, finally, do we yet know how long a stream from an infected surface, or sub-soil, should be prohibited as a water supply.

Comparison of Death-rates.

In conclusion, if we review the sanitary history of Belfast we find that further light is thrown on the typhoid problem by contrasting the record of the general death-rate and the zymotic death-rate with that of deaths due to typhoid fever.

In 1889, Belfast, in common with other places, suffered from an outbreak of typhoid fever, and, though the outbreak subsided in a measure, there was still in subsequent years an excessive amount of typhoid. This condition of things remained until 1897, when a violent epidemic occurred, the causation of which I have discussed in detail. It is, therefore, clear that typhoid cases have been in excess in Belfast since water was supplied from the typhoid-infected district of Stoneyford. Whatever deficiencies have been shown to exist in the sanitation of the

Typhoid Fever in Belfast

city, it has also been made clear that a great degree of reform has been carried out. Not only have important regulations been enforced but a great amount of insanitary property has been swept away. Nor has this sanitary reform been without a very distinct effect on the death-rate of the city. The following tables show this clearly:

Return, showing annual death-rate from Typhoid Fever, Zymotic Diseases, and total from all causes, during the years 1881—1902.

Year	Typhoid Fever, per 10,000	Zymotic Diseases, per 1,000	All Causes, per 1,000	Remarks
1881	3·7	2·4	23·6	
1882	2·9	4·0	25·8	
1883	2·8	4·3	26·2	
1884	2·4	2·9	23·4	
1885	2·2	5·3	27·9	
1886	3·9	2·4	23·7	
1887	3·5	4·0	25·9	
1888	3·3	3·4	25·3	Main Drainage Works commenced, and Stoneyford Water introduced.
1889	7·9	3·9	25·8	Dairy Regulations came into force.
1890	7·6	5·2	29·5	Bye-Laws as to New Buildings came into force, and Smoke and Water test commenced by Public Health Department.
1891	5·9	2·6	25·5	
1892	4·1	3·8	26·5	Systematic removal of refuse introduced.
1893	4·4	4·7	25·8	
1894	5·1	4·1	23·2	Bye-Laws for the Regulation of Piggeries came into force. Commenced testing drainage of all new houses having internal water-closets. Disinfecting Station established.
1895	6·2	3·3	24·3	
1896	4·5	3·3	23·1	Main Drainage Works completed.
1897	11·4	3·6	23·0	Infectious Disease (Notification) Act adopted. Typhoid Fever at Stoneyford Catchment Area.
1898	18·8	3·8	22·8	Commenced testing new house drains of every description.
1899	7·5	2·9	22·7	Parliamentary powers obtained to substitute water-closets for privies. Typhoid Fever at Stoneyford Catchment Area.
1900	7·2	2·1	21·3	Typhoid Fever at Stoneyford Catchment Area.
1901	9·7	3·3	22·4	Do.
1902	4·7	2·8	20·8	Do.

The general death-rate has fallen continuously since 1890. If we compare the death returns for the eight years between 1881—1888 with those of the eight years 1895—1902, we will be able to observe at

a glance the effect on the health of the city due to improved sanitation. The returns are in each case per 1000 of the population.

Years	Total death-rate	Zymotic death-rate	Typhoid death-rate	Zymotic death-rate apart from typhoid
1881—1888	25·2	3·59	·31	3·27
1895—1902	22·5	3·14	·87	2·27

From this it is clear that the sanitary rearrangement of the city has not resulted in failure, and, while the general death-rate has decreased, the zymotic death-rate, apart from typhoid, has decreased to a much greater extent. There has, however, been conspicuous failure in relation to typhoid fever, the death-rate from which has risen to an extraordinary degree.

Conclusions.

To sum up, the facts demonstrate the existence of a special source of typhoid infection which has not been removed by the sanitary reforms that have lowered the general death-rate and the zymotic death-rate. The investigation has failed to discover this source of infection in the city. On the other hand the Stoneyford catchment area has contained a focus of typhoid infection, which has there remained unaltered during the period of sanitary reform in the city, and we are compelled to hold it to be the primary cause of the excessive amount of typhoid fever which has existed in Belfast since water from this source was supplied to the citizens.

A METHOD OF PRODUCING CHROMATIN STAINING IN SECTIONS.

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IN a recent Report⁽¹⁾ upon the new parasitic bodies associated with tropical splenomegaly, Lieut. S. R. Christophers, I.M.S., published a method of producing chromatin staining in sections. This method, however, labours under the disadvantage that, after staining, the section must be dried in the air before it is cleared and mounted, since the chromatin reaction is lost if dehydration by absolute alcohol is attempted. The consequent shrinkage of the tissue, even in very thin sections, renders the method of little service for delicate work.

Prior to this I had made many futile attempts to produce this reaction in sections by employing my modified Romanowsky stain⁽²⁾ and had come to the conclusion that the processes of hardening and embedding tissues for section cutting had induced chemical changes in the tissues which interfered with the reaction. Christophers' method, however, showed that this was not the case, and my further experiments have resulted in a certain measure of success, inasmuch as the method described below induces a chromatin staining of the tissues which resists the decolorising action of the absolute alcohol used for dehydration. There is, further, a certain convenience in the employment of a single staining fluid instead of separate solutions of eosin and 'ripened' methylene blue. The method in question is probably susceptible of further improvement and simplification, but, as some such method is much wanted in several branches of research work, it may at least be of temporary service.

Small pieces of tissue, after a short sojourn in spirit followed by absolute alcohol, are put through xylol and paraffin in the usual way—adherence to the narrow time limits given by Christophers is not essential. Sections as thin as are consistent with the nature of the

tissue are to be recommended, those which I employ are of an average thickness of 5μ . After fixation on a perfectly clean slide, by pressure, drying and heat, especial pains must be taken to dissolve out all the paraffin; this is secured by applying xylol while the paraffin is still liquid and by treating the section with alternate baths of absolute alcohol and xylol 3 or 4 times. After the final bath of alcohol, distilled water is poured on to the slide before the alcohol has evaporated and the section is well washed to remove all traces of the alcohol. The excess of water on the slide is now got rid of by blotting with tissue paper and, while the section is still moist, a drop or two of fresh blood serum is placed on it and allowed to soak into it for five minutes. The action of the serum I conceive to be of the nature of a 'refreshing' of the tissue, probably in the direction of a restoration of its normal alkalinity. The excess of serum is now removed by blotting and the remainder allowed to dry as a thin film on and around the section.

The staining fluid is now prepared by mixing 2 parts of my Romanowsky stain with 3 parts of distilled water. This mixture is poured on the section, the slide covered with the lid of a Petri dish and staining is allowed to go on for 1 to $1\frac{1}{2}$ hours. I find the best results are obtained by pouring off the stain once or twice during this period and replacing it with a fresh supply.

The stain is finally washed off with distilled water and the section examined under a low power when the cell nuclei should appear very densely stained, in fact almost black. It will be found that the section and the part of slide covered by the serum are quite free from the deposit which so often results from prolonged Romanowsky staining.

The subsequent processes of decolorisation and differentiation are essentially the same as those which I have employed in ordinary section staining for the last year and of which I recently published the details⁽³⁾. Two solutions are required, 1-1500 acetic acid and 1-7000 caustic soda, freshly prepared with distilled water. The alkaline solution is employed to dissolve out from the tissue the excess of eosin, which would otherwise render it too opaque, besides forming a bad contrast colour to the red chromatin. The acid solution removes the excess of blue and at the same time brightens the red tint of the chromatin. These two solutions are used alternately, commencing with the acid, and the section is frequently observed under a low power until the desired colour contrasts are obtained. As a rule the acid solution is that which requires to be most used. When the cell nuclei of the tissue are seen to be of a deep Romanowsky red colour, and the rest of the tissue

either a very pale pink or a light blue, the section is washed in water and is ready for dehydration by absolute alcohol. This process is conducted very rapidly, and, to this end, the excess of water on and around the section is removed, by blotting with tissue paper and wiping, so that as little work is left for the alcohol as possible. A few drops of alcohol are allowed to run down the slide and over the section, the slide being then at once dipped in xylol and washed therein until the section is perfectly cleared. It is now mounted in Canada balsam which is dropped on when the xylol is on the point of evaporating.

The material employed in testing the above method consisted of the organs of rats and rabbits infected with *Trypanosoma brucei*, and in sections of these organs, stained as above, the parasites are readily recognisable by reason of the deep red staining of the macro- and micro-nuclei, while the bodies of the parasites are frequently coloured a pale blue; the flagella are unstained. The bright red reaction of the cell nuclei before dehydration may be modified to a purplish tint by the action of the alcohol, but this is of only secondary and aesthetic importance as the chromatin of the parasites is unaffected. In the same sections stained by many other methods no trace of the Trypanosomata could be detected.

A little experience is necessary in controlling the effects of the acid and alkaline solutions, but, once this is attained, and provided that the initial staining has been carried to a sufficient degree of density, the method gives constant and regular results.

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FURTHER OBSERVATIONS ON THE DIAGNOSIS OF ANKY-
LOSTOMA INFECTION WITH SPECIAL REFERENCE TO
THE EXAMINATION OF THE BLOOD.

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THE experience of the last few years in the Westphalian coal-field has shewn that the presence of *Ankylostoma* in a mine may become a matter of considerable industrial importance. Since it has been shewn that several of the metalliferous mines in Cornwall are thoroughly infected, the question of how far it may be present in, or spread to, other British mines has arisen in a practical form. When any large number of individuals have to undergo a medical examination for any such purpose it is of importance that such examination should be convenient at once for the examiner and for the examinee, that it should occupy as short a time as possible, and should, at the same time, be reliable. The diagnosis of the presence of *Ankylostoma* is commonly made by searching for the characteristic eggs in the faeces; and indeed a final diagnosis must rest in nearly every case on the results of this procedure, which is therefore not easily replaced by any other method when separate individuals are in question. But as applied to a large

body of active working men this process has many disadvantages, and at the request of the Home Secretary an inquiry has been made into the practicability of other methods of examination¹.

The means of diagnosis at our disposal may be grouped under three heads :—

- (1) General inspection of employees for cases of anaemia.
- (2) Microscopical examination of faeces.
- (3) Examination of the blood.

It will be convenient to examine each of these methods in order.

(1) *General Inspection.* From the point of view of Preventive Medicine, great emphasis must be laid on the fact that many, if not the majority, of the individuals who harbour the parasite in their intestines do not present any objective signs of disease, nor do they complain of any symptoms of illness. On the contrary, they will appear on ordinary examination, and will profess themselves to be, in perfect health. Yet it is these healthy "worm-carriers" who constitute the greatest danger of ankylostomiasis as a place disease; they have indeed greater powers than those who are actually ill of carrying infection from place to place, owing to their very healthiness and ability to work. Thus if one looks over a shift of men at Dolcoath, one sees comparatively few cases of obvious anaemia, even if the lips and conjunctivae are carefully examined and ruddy cheeks disregarded. If accurate haemoglobin estimations are made in these men, one finds an undue number of cases where the blood is on the lower limit of health (about 90 p.c. Hb.), and a certain number still lower (70 p.c. and upwards). But, though we have been mostly concerned with those who are really anaemic, we have found plenty of men with 95—105 p.c. Hb., examination of whose faeces shewed that they were infected. It is true that in any large collection of infected men a certain number of individuals will generally be found who are pale, complain of dyspnoea, and shew other signs of anaemia. But this is not invariably the case. At East Pool Mine (see below p. 451) I could not hear of any suspicious symptoms among the men at the time of my visit, nor could I find an underground worker who was anaemic on superficial examination. Yet *Ankylostoma* eggs were found in several samples of faeces, and there are reasons for thinking that some two-fifths of the men are probably infected.

It is, then, useless when searching for *Ankylostoma* in a mine to give any weight to the absence of cases of anaemia among the men,

¹ A short account of the results obtained has already appeared as a Parliamentary Paper [Cd. 2066], 1904.

though the presence of such illness would of course be very suggestive.

(2) *Microscopical Examination of Faeces.* A mine may be examined for the presence of the worm by obtaining faeces from a number of the men and submitting these to microscopical examination. Results of a very definite character are thus obtained at once. There are, however, several practical objections to this method:—

(a) It must be remembered that the men are not under control in hospital, and that the samples are furnished by their goodwill alone. While paying the highest tribute to the willing way in which the great majority of the miners who have been asked to do so have assisted in the inquiry by performing what cannot be to them a pleasant task, I have no doubt that many refusals will often be met with. This is especially to be expected in places where there are no cases of actual illness in which the men's active interest may be aroused.

(b) In any case, it takes at least several days to obtain a sufficient number¹ of samples of faeces.

(c) There is a great opportunity for fraud, unless the stools are passed under supervision; this is done in Westphalia, but would hardly be possible in this country.

(d) The microscopical examination is simple, and in most cases where eggs are present they are in such numbers that they are found at once. It is, however, necessary to search for a long time and on more than one occasion, before giving a definite verdict that eggs are absent.

(e) There is no doubt that a certain number of cases occur in which, though worms are present in small numbers (as proved by autopsy), the eggs are not found by direct microscopical examination of the stool. Nor are eggs found till about a month or six weeks after the larvae have entered the bowel. The results obtained by this method of examination are not therefore absolutely infallible.

Instead of obtaining stools directly from the men, one may collect

¹ What proportion of the total number employed underground constitutes a "sufficient number" is difficult to say. In most cases, however, where the worm has gained a footing in a mine, a large proportion—often nearly the whole—of the men are infected. The proportion, no doubt, varies with the suitability of the conditions (temperature, moisture, etc.) for the abundant growth and spread of the larvae; it will be lower in cool and dry than in hot and wet mines. If these conditions are not unfavourable, an examination of 10 per cent. of the men, taken at random, will probably give a very good idea of the frequency of infection. Scattered cases, however, might easily be missed in this way.

portions of any faecal deposits which may be found underground. I have recently examined a large number of such specimens, and find that valuable results may be in this way obtained. Most of the objections mentioned above cease to apply when this method is used, but some other difficulties arise—

(a) In the first place it is a grave objection that the individual origin of each specimen is unknown.

(b) If circumstances are favourable, nearly all the *Ankylostoma* eggs present will have hatched out into larvae. The empty eggshells are practically invisible, and it is impossible to identify the larvae with certainty. A few dead eggs remain for a long time in such a condition that their nature is clearly seen, but they may be so few in number, in a stool which previously contained an abundance, as to require a very prolonged search to find them.

(c) Animals foreign to the human intestine enter the faecal deposits from the earth. Certain small nematode worms, which have their natural habitat in moist earth, are very often present; their eggs and larvae in some cases are not at all unlike those of *Ankylostoma*, and might easily cause confusion.

(d) It is not always an easy matter to obtain any number of specimens underground, and it is to be hoped that in the future it will be impossible.

(3) *Examination of the Blood.* Estimations of haemoglobin are often of great value in determining the presence or absence of an actual anaemia, allowing 90 p.c. of Hb. (on the scale of the Gowers-Haldane instrument) as the lowest limit which can be passed without comment.

The blood changes in those cases of *Ankylostoma* infection and of ankylostomiasis which were first examined in Cornwall have been already described in some detail¹, and it was shewn that in nearly every case a marked increase in the eosinophile leucocytes was present. This phenomenon seemed at once so frequent and so simple of demonstration that a further enquiry into the possibility of using it as a practical test on a large scale was thought to be desirable. Control examinations of the blood of non-infected miners were also necessary. The investigation has been limited to estimations of the *percentage* number of eosinophiles in dry blood films; any attempt to ascertain the *absolute* eosinophiles (which is doubtless the really important point) would necessarily remove the method from the sphere of simple practical hygiene. At each mine which was visited a number of men were examined, and a collection

¹ *Journal of Hygiene*, III, 1903, p. 111.

of blood films made; at the same time specimens of faeces were, if possible, obtained from the underground workings. In this way a quantity of material could be obtained in a short time and examined at leisure in more suitable surroundings.

The data which have in this way been obtained are set out below; a short account of the conditions under which each group of men work precedes an account of the blood examinations and a summary of the evidence obtained from the examinations of faeces as to the presence of intestinal parasites. At the end of the detailed statement the figures are collected and compared; but the main result may be here anticipated. It is that the great majority of those who harbour *Ankylostoma* shew an eosinophilia, while non-infected miners do not.

A. Miners infected with *Ankylostoma*.

Dolcoath Mine, Cornwall.

The physical conditions of this mine have been already fully described: briefly, it is a deep, hot, wet, tin mine; faecal contamination previously very bad, but now much improved: Haldane, R. A. Thomas and myself have found *Ankylostoma* eggs in the faeces of every regular underground worker who has been examined, and there is every reason to suppose that practically everyone is infected. *Trichocephalus* is very common and a few have *Ascaris*.

i. *Mén actually known to have eggs in faeces.* The majority of the differential counts of this group have already been published (this *Journal*, III. 1903, p. 128). Since that time¹ I have been enabled to examine films from a number of fresh cases. The whole number now amounts to 61 men; 51 of these shew an eosinophilia of more than 10 p.c., while only two are under 5 p.c.: average 17·8 p.c.

ii. *Underground workers at Dolcoath whose faeces have not been examined but who may be assumed to have Ankylostoma.*

(a) In 1902 twenty-two such cases were examined: most of the counts have been already given. They were mostly men who complained of some, but not extreme, symptoms of anaemia. Of these 20 were over 10 p.c.; average 19·7 p.c.

¹ This extension of the cases, and especially the periodical examinations of the blood in men under treatment, has been rendered possible only by a copious supply of films and estimations of haemoglobin for which I am indebted to Mr R. A. Thomas.

(β) December, 1903: 24 men from the day shift, none of whom were obviously anaemic, taken at random in the "dry" (changing house): 16 over 10 p.c.; average 13.4 p.c. The detailed counts are as follows:—

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
W. J. G. ¹	20.6	25.4	2.6	45.8	3.6	2.0
J. J.	36.4	8.2	1.6	48.8	4.4	0.6
J. L.	26.6	17.4	1.4	48.4	5.6	0.6
J. S.	15.4	13.0	0.6	62.4	8.0	0.6
J. C. M.	26.4	19.2	3.4	41.6	8.2	1.2
C. M.	15.8	27.2	0.8	46.8	8.8	0.6
G. G.	28.4	18.8	2.4	40.4	9.6	0.4
C. B. W.	22.4	17.2	2.0	48.0	9.6	0.8
—, C.	17.6	10.4	2.4	58.4	10.0	1.2
J. H. S.	29.0	17.8	2.0	39.8	10.0	1.4
J. W.	17.2	20.8	0.8	50.0	10.8	0.4
F. C.	26.4	12.4	2.2	47.6	11.0	0.4
R. H. W.	15.4	10.6	1.4	60.0	11.8	0.8
F. P.	33.4	7.6	2.6	43.2	11.8	1.4
J. H.	21.2	8.8	1.6	54.8	12.0	1.6
W. H. G.	16.0	21.4	2.8	46.0	13.0	0.8
W. G.	14.8	15.8	1.6	53.8	13.2	0.8
J. V.	34.8	7.6	1.2	40.2	15.2	1.0
H. M.	27.6	16.0	4.8	35.4	15.4	0.8
F. P.	27.4	5.8	0	47.8	18.0	1.0
J. C.	22.2	20.4	0.8	34.8	21.6	0.2
E. J. B.	18.6	10.4	1.2	47.2	21.6	1.0
T. G.	11.8	6.0	0.8	48.8	31.0	1.6
A. M.	27.4	10.8	1.0	28.2	31.6	1.0

(γ) February, 1904: a similar series taken from 41 men of the night shift at random in the "dry": 36 over 10 p.c.; average 20.7 p.c. None of the men complained of any anaemic symptoms:

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
C. S.	31.4	6.2	1.2	56.8	3.8	0.6
—, H.	19.0	7.6	4.4	60.0	8.0	1.0
A. B.	25.8	6.6	0.8	57.2	8.6	1.0
J. W.	19.4	3.8	0.2	67.4	8.8	0.4
A. O.	32.4	5.6	2.2	49.2	9.2	1.4
R. S.	34.0	7.6	3.6	44.0	10.2	0.6
S. A.	24.2	9.0	4.4	50.4	11.0	1.0
—, S.	25.0	14.2	4.6	44.6	11.2	0.4
—, F.	18.4	3.2	1.8	64.0	12.0	0.6
J. H.	30.2	10.6	2.4	44.0	12.4	0.4

¹ The counts in these tables are all based on the classification of 500 leucocytes; in a few cases 1000 were enumerated.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
W. P.	18.0	6.8	4.0	58.4	12.6	0.2
G. B.	36.0	5.2	0.8	43.0	13.8	1.2
J. U.	32.2	6.2	3.2	43.0	14.8	0.6
— B.	16.6	4.2	2.0	62.0	15.0	0.2
P. C. B.	21.4	4.4	2.0	56.4	15.2	0.6
R. O.	26.4	8.8	1.2	46.8	16.0	0.8
W. T.	36.6	6.6	1.6	38.6	16.4	0.2
R. M.	34.0	7.0	1.4	39.8	16.8	1.0
W. P.	24.4	12.2	4.0	41.8	16.8	0.8
T. S.	24.8	5.6	2.0	49.2	17.6	0.8
E. I.	29.6	4.4	0.4	47.2	18.2	0.2
W. W.	31.6	4.6	0.8	43.8	19.0	0.2
J. T.	22.4	6.0	3.0	47.6	20.6	0.4
T. D.	32.6	7.2	2.4	36.0	20.6	1.2
S. C.	15.8	8.4	3.4	50.6	20.8	1.0
T. H. B.	27.0	8.8	0.6	42.0	21.2	0.4
F. P.	33.8	3.0	2.0	37.6	21.6	2.0
J. B.	26.0	5.2	3.0	42.0	22.2	1.6
T. W.	34.0	4.6	1.2	36.2	22.4	1.6
B. P.	31.2	4.6	1.2	36.0	25.0	2.0
R. M.	35.2	7.8	0.6	30.2	26.0	0.2
F. C.	19.4	5.0	2.2	45.4	27.8	0.2
J. C.	21.6	2.8	2.2	44.6	28.2	0.6
P. E.	23.6	6.2	2.4	37.6	29.6	0.6
R. O.	24.2	4.2	2.4	36.6	32.0	0.6
E. T.	16.2	5.4	0.8	44.0	33.0	0.6
T. C.	18.4	5.8	2.0	37.8	34.4	1.6
— V.	26.2	3.4	0.6	30.8	38.2	0.8
J. C.	26.8	4.6	0.6	25.0	42.8	0.2
A. H.	23.8	5.4	2.4	24.4	43.8	0.2
A. E.	12.6	2.0	0.6	32.8	51.6	0.4

This series and the last (β and γ) contain four counts with low (3.6 to 5.6) percentages of eosinophiles. It has unfortunately not been possible to obtain faeces from these men, and they may not harbour the worm at all. But there is a probability that they do.

The next table summarises the figures obtained from this series of infected miners¹—

¹ At Dolcoath the total number of underground hands ("complement") is 717, of whom 148 have been examined = 20 per cent.

Percentages of Eosinophiles.

	Cases	<1	1—	2—	3—	4—	5—	6—	7—	8—	9—	10—	15—	20—	25—	>30	Av. per- centage
I. Known to be infected	61				2		1	1	1	1	4	18	15	10	3	5	17·8
II. Believed to be infected																	
(a) Dolcoath, 1902	22										2	5	7	3	0	5	19·7
(β) „ Dec. 1903	24				1	1	1			3	2	9	3	2	0	2	13·4
(γ) „ Feb. 1904	41				1					3	1	8	9	7	5	7	20·7
Total	87				2	1	1			6	5	22	19	12	5	14	18·5
Total of I. and II.	148				4	1	2	1	1	7	9	40	34	22	8	19*	18·2

* Of these, 12 are between 30 and 40 p.c., and 3 between 40 and 50 p.c. : the others are: 51·6, 56·2, 66·2 and 72·7 p.c.

B. Non-infected Mines.

a. *Talke o' th' Hill, N. Staffs.*; coal-pit: temperature at working face 72°—80° F. No history obtainable of any illness like ankylostomiasis: no cases of obvious anaemia seen. No traces of *Ankylostoma* or other worms found in faeces collected underground: faecal contamination not very obvious. Films obtained from 40 men: 38 were under 5 p.c.; average 1·9 p.c. Underground complement 480 = 8 p.c. examined.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
W. F.	38·2	9·6	1·2	50·2	0·2	0·6
J. R.	29·4	5·6	0·8	64·0	0·2	0 ¹
W. D.	34·8	6·6	2·4	55·4	0·4	0·4
E. F.	25·2	7·2	2·8	63·6	0·4	0·8
I. F.	26·6	7·6	2·2	62·2	0·4	1·0
T. W.	12·8	2·6	1·0	82·6	0·4	0·6
W. E.	13·6	2·2	1·0	82·6	0·6	0 ¹
D. E.	29·6	4·8	2·6	61·8	0·6	0·6
J. W.	28·6	7·6	0·4	62·2	0·6	0·6
G. R.	28·8	6·6	1·4	62·4	0·6	0·2
W. B.	36·2	5·4	1·6	55·6	0·8	0·4
A. S.	26·6	9·8	3·2	59·0	0·8	0·6
J. F.	22·6	6·4	1·4	68·4	0·8	0·4
C. F.	30·8	8·2	1·8	58·0	1·0	0·2
W. S.	24·8	4·2	1·2	68·0	1·0	0·8
F. C.	25·4	3·8	1·8	67·0	1·0	1·0
T. W.	34·4	6·6	1·6	55·4	1·0	1·0
W. L.	53·2	8·0	1·2	35·8	1·2	0·6

¹ Mast cells present, but not seen in the 500 cells enumerated.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
W. R.	28.0	7.6	5.8	56.4	1.4	0.8
W. P.	45.4	3.8	0.8	47.4	1.6	1.0
E. B.	43.4	8.4	3.0	43.0	1.6	0.6
W. G.	25.6	7.6	2.6	61.6	1.8	0.8
J. M.	29.0	4.4	3.2	61.0	1.8	0.6
T. W.	24.0	5.4	3.4	64.8	2.0	0.4
G. B.	24.0	10.4	5.2	56.2	2.2	2.0
A. F.	29.8	11.2	1.0	54.8	2.2	1.0
W. J.	48.4	5.6	2.0	41.0	2.2	0.8
A. N.	22.4	6.4	3.0	65.4	2.2	0.6
J. W. L.	35.0	8.4	2.4	50.4	2.6	1.2
G. W.	31.0	6.6	3.4	55.8	2.8	0.4
M. K.	23.6	5.6	3.2	64.2	2.8	0.6
A. L.	23.0	12.2	5.2	54.6	3.4	1.6
V. W.	36.4	5.8	1.6	52.0	3.4	0.8
A. D.	18.6	10.0	8.2	59.0	3.8	0.4
J. W.	27.6	4.0	0.6	63.2	3.8	0.8
J. W.	39.0	4.8	0.6	51.6	3.8	0.2
H. B.	26.6	10.8	4.0	54.2	4.0	0.4
H. F.	22.4	4.8	0.6	66.8	4.6	0.8
R. H.	28.6	13.4	6.2	46.0	5.0	0.8
E. T.	21.0	2.6	2.0	67.8	6.0	0.6

β. Snailbeach, Shropshire: lead and zinc mine; temperature 65° to 68° F. Nothing found or heard suggestive of *Ankylostoma*; 22 lots of faeces examined. No other intestinal parasites found. Faecal contamination very bad, and levels mostly wet, though the ladders and upper travelling roads were dry. Films from 42 men, 38 of which were under 5 p.c.; average 2.1 p.c. Underground complement 70 = 60 p.c. examined.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
J. C.	8.6	12.0	3.0	76.4	0 ¹	0 ²
W. P.	7.4	23.4	2.0	66.8	0.2	0.2
E. A.	15.2	11.2	3.8	68.8	0.4	0.6
J. E.	20.8	11.8	3.0	63.8	0.4	0.2
A. M.	15.0	10.2	3.4	68.8	0.4	2.2
R. P.	17.6	21.6	3.0	57.0	0.6	0.2
E. C.	11.2	20.2	1.6	65.2	0.8	1.0
G. P.	18.4	16.8	2.8	60.8	1.0	0.2
G. L.	36.6	15.4	2.8	43.0	1.0	1.2
G. H.	19.6	20.6	1.8	56.0	1.0	1.0
J. G.	19.4	11.8	1.4	66.0	1.0	0.4
J. B.	14.8	13.8	2.2	67.2	1.2	0.8
W. P.	24.8	11.0	2.4	60.2	1.2	0.4

¹ Eosinophiles present, though not seen in the 500 cells enumerated.

² Mast cells present.

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	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
W. M.	21·8	17·2	3·2	56·0	1·2	0·6
R. P.	18·2	15·0	0·6	64·0	1·2	1·0
F. B.	26·4	10·6	3·0	58·2	1·4	0·4
J. G.	22·0	16·6	0·2	59·2	1·4	0·6
W. B.	15·6	14·6	2·2	65·6	1·4	0·6
J. P.	16·0	19·0	4·0	59·2	1·4	0·4
M. R.	15·6	25·8	1·8	54·8	1·6	0·4
J. H.	15·2	27·4	3·0	52·0	1·6	0·8
W. H.	22·6	32·2	3·8	39·4	1·6	0·4
J. G.	15·2	21·8	3·0	57·4	1·6	1·0
J. H.	20·6	12·4	1·4	63·6	1·6	0·4
R. J.	15·2	14·0	3·0	65·2	1·8	0·8
T. P.	28·6	12·2	2·4	53·8	2·0	1·0
J. P.	23·4	10·2	1·0	63·0	2·0	0·4
I. B.	24·2	17·4	2·6	52·2	2·2	1·4
J. H.	9·6	21·6	2·4	63·0	2·2	1·2
J. R.	16·0	23·2	1·4	55·8	2·4	1·2
J. B.	15·6	17·2	1·4	63·2	2·6	0 ²
P. P.	24·0	16·8	1·0	55·0	2·8	0·4
E. P.	17·0	23·4	5·0	50·0	3·2	1·4
M. H.	38·2	14·6	0·6	42·4	3·4	0·8
A. P.	16·4	25·6	2·6	50·6	3·4	1·4
J. J.	15·0	19·6	1·4	59·2	3·6	1·2
T. S.	9·8	22·2	1·8	61·0	3·8	1·4
—, P.	6·8	19·2	3·0	66·6	4·0	0·4
E. P.	12·6	10·6	0·8	69·0	5·2	1·8
R. L.	39·6	13·2	2·4	37·4	6·0	1·4
W. H.	20·2	14·8	0·4	56·4	6·8	1·4
E. H.	17·8	23·8	1·0	49·6	7·2	0·6

γ. *Birchenwood, N. Staffs.*; coal-pit; temperature 72°—80° F. at face. Nothing found or heard suggestive of *Ankylostoma*; faecal contamination not extensive. 12 films, 9 of which were under 5 p.c.; average 2·7 p.c.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
C. C.	18·4	10·0	2·8	66·8	0·8	1·2
S. B.	26·4	7·8	2·0	62·8	1·0	0 ²
F. C.	35·0	6·0	1·2	55·4	1·0	1·4
G. H.	37·2	6·0	1·0	54·2	1·0	0·6
A. S.	30·4	12·6	2·6	52·0	1·6	0·8
L. B.	25·2	6·6	1·2	64·4	1·6	1·0
M. C.	49·8	6·8	0·8	40·6	2·0	0 ²
S. W.	29·6	5·8	0·4	61·2	2·6	0·4
E. W.	35·6	5·8	1·8	53·2	3·4	0·2
I. C.	43·2	4·4	1·2	45·8	5·4	0 ²
J. K.	20·8	9·8	1·0	61·6	5·8	1·0
J. M.	27·8	7·2	0·6	57·6	6·0	0·8

² Mast cells present.

δ. *West Kitty, St Agnes, Cornwall*; tin mine; shallow (110 fathoms) and quite cool. No history of anaemia etc. obtainable. Ten films, nine of which were under 5 p.c.; average 2·6 p.c.; the tenth case shewed 23·8 p.c. and on further examination abundant eggs of *Trichocephalus* and *Ankylostoma* were found in his faeces.

ε. *Surface workers at Dolcoath*. Though not strictly speaking miners, an examination of these men was made in order to exclude the Camborne climate and contact with Dolcoath ore as possible factors in the production of an eosinophilia. The men selected were tin-dressers, who only came in contact with the material after it had been passed through the stamps and had been roasted. Films 14, all of which were under 5 p.c.; average 2·0 p.c. There is no history of any of the purely surface workers at Dolcoath ever having had any anaemic symptoms.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
M. M.	19·2	20·8	1·8	57·4	0·2	0·6
T. C.	20·0	20·4	1·4	57·4	0·2	0·6
W. W.	15·0	27·4	3·0	53·6	0·6	0·4
J. P.	27·4	11·8	3·6	56·0	1·0	0·2
J. R.	21·6	5·0	0·6	71·0	1·2	0·6
R. G.	7·0	4·4	5·4	81·6	1·2	0·4 ¹
W. W.	16·8	25·4	1·6	53·8	1·4	1·0
J. C. P.	12·8	7·6	0·6	76·4	2·2	0·4
R. L.	42·0	12·0	2·4	40·0	2·4	1·2
J. C.	22·8	15·6	1·0	56·4	3·2	1·0
W. P.	35·0	18·6	2·2	40·4	3·2	0·6
W. H. R.	35·2	12·2	2·0	46·8	3·2	0·6
W. R.	19·8	16·2	3·4	55·6	4·0	1·0
R. P.	33·6	3·6	3·4	54·4	4·8	0·2

ζ. *Levant Mine, St Just, Cornwall*; tin, copper, and arsenic. A special interest attaches to this mine, which will perhaps justify a rather more detailed account of the results obtained. The present workings are to a large extent under the sea; the ventilation is very imperfect and the temperature very high, rising from 76° at the bottom of the downcast shaft (about 270 fathoms) to 90—93° in large areas of the deeper workings (300—320 fathoms). The level, ladders, etc. are wet and muddy and faecal contamination extensive. A considerable number of men have returned to work here in recent years from infected districts abroad, and I was aware that a few men from Camborne, who were actually known to harbour the worm, had been employed underground. I visited the mine, therefore, in the expectation that ankylostomiasis would be

¹ Considerable leucocytosis present from cystitis due to an enlarged prostate.

found to be prevalent. Such, however, was not the case. No history of any suggestive illness among the men could be obtained: the mine has indeed a local reputation for being more healthy to work in than others in the neighbourhood¹. From the underground workings 25 samples of faeces were obtained: these shewed the presence of numerous intestinal parasites (*Trichocephalus* in 24, *Ascaris* in 3, *Oxyuris* in 1) but no trace of *Ankylostoma*. Blood films were obtained from 41 men²; the differential counts of these gave the following figures:

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
W. D.	22·8	15·2	3·6	56·8	0·4	1·2
W. C.	31·2	15·6	0·8	51·6	0·4	0·4
W. S.	31·0	18·6	1·0	48·2	0·8	0·4
J. W.	29·8	10·0	0·4	58·8	0·8	0·2
H. M.	31·0	11·2	0·6	55·2	0·8	1·2
F. W.	23·0	19·0	2·6	53·4	1·0	1·0
—, W.	36·6	11·0	0·8	50·2	1·2	0·2
T. E.	29·0	7·6	0·4	61·0	1·2	0·8
W. T.	21·0	25·0	1·0	50·2	1·4	1·4
J. F.	42·2	7·6	0·4	47·4	1·4	1·0
J. T.	20·6	14·8	3·8	58·4	1·4	1·0
J. R.	32·6	8·8	0·4	55·8	1·6	0·8
J. H. T.	35·6	10·0	0·4	51·8	1·6	0·6
W. J. G.	35·4	6·8	0·6	54·8	1·8	0·6
H. J.	45·2	4·4	1·2	46·4	2·0	0·8
R. E.	25·4	8·0	0·6	63·6	2·2	0·2
T. M.	29·0	29·2	0·8	38·4	2·2	0·4
J. L.	30·0	10·6	0·8	54·6	2·2	1·8
W. T.	25·2	12·2	3·0	56·8	2·4	0·4
G. A. M.	33·6	12·0	2·6	49·0	2·4	0·4
R. W.	19·8	8·4	2·0	66·6	2·6	0·6
W. L.	34·4	18·4	1·8	42·2	2·6	0·6
J. R.	28·6	15·4	1·2	51·2	2·8	0·8
S. T.	40·0	8·6	1·2	46·0	2·8	1·4
R. O.	31·6	3·4	0·4	60·4	2·8	1·4
J. P.	35·8	8·6	1·0	50·4	2·8	1·4
W. F. M.	30·6	8·4	1·6	55·4	3·0	1·0
S. A.	15·6	17·8	3·4	59·6	3·0	0·6
—, T.	19·4	18·8	2·4	55·6	3·2	0·6
J. N.	48·8	10·2	0·2	36·6	3·4	0·8
B. P.	38·4	4·6	0	52·6	3·8	0·6
W. E. L.	39·2	6·8	0·8	48·8	3·8	0·6
F. U.	37·4	8·8	0·8	48·0	4·2	0·8
R. T.	36·4	10·0	0·4	48·6	4·4	0·2

¹ All these neighbouring mines (Botallack, etc.) have shut down some years since.

² Complement 478: examined 8½ per cent.

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
T. M.	32·6	14·6	2·8	44·4	4·6	1·0
J. E.	27·4	14·0	1·4	52·0	4·8	0·4
A. W. K.	16·6	30·4	3·2	44·6	5·0	0·2
R. H.	34·0	6·8	1·2	51·4	6·4	0·2
J. R.	21·0	7·8	0·6	61·4	8·4	0·8
H. T.	22·4	7·8	0·4	45·2	23·8	0·4
J. M.	21·8	8·0	0·4	42·0	25·6	2·2

Of these 41 men, 36 counts were thus less than 5 p.c.; of the rest two reached the very suggestive figures of 23·8 and 25·6. Samples of faeces were obtained by Dr C. S. Jago from the men with the four highest counts; and six weeks later I saw three of the men again and obtained further films. The results were as follows:

	p.c. of Eosin's	First Examination Stools	Second Examination Stools	Hb	p.c. of Eosin's
R. H.	6·4	<i>Trichocephalus</i>	—	104 p.c.	7·2
J. R.	8·4	<i>Trichocephalus</i> & <i>Oxyuris</i>	—	—	—
H. T.	23·8	<i>Trichocephalus</i> & <i>Ascaris</i>	<i>Trich. & Ascaris</i>	96	17·0
J. M.	25·6	<i>Trichocephalus</i> ¹	<i>Trich. & Ascaris</i>	105	18·4

No traces of *Ankylostoma* were found, and, since practically every underground worker in Levant seems to harbour *Trichocephalus*, the two highest figures were doubtless due to the *Ascaris* infection. The other two may be cases of *Trichocephalus* eosinophilia. I could find no other cause in R. H., who is a very robust lad.

The average for these 41 cases is 3·7 p.c.; without the two highest counts it is only 2·6 p.c.

Ankylostoma thus appeared to be entirely absent from a mine which seemed to be situated most favourably for its introduction and spread. The reason for this was, on further investigation, found to lie in the nature of the water which permeates the mine. Some of this flows in from the land and is ordinary spring water, but most of it is derived from the sea by leakage through the roof into the upper levels. Seven samples of water from different places were examined as to their content in salt. The results were as follows:

¹ Repeated searches failed to find any *Ascaris* eggs in this first sample; in the second they were very abundant.

						NaCl per cent.
A.	210 fathoms level : dripping through from 190	2.42
B.	210 further West standing water	2.44
C.	230 on North side	2.72
D.	230 on South side	2.28
E.	230 on East (landward) side	0.94
F.	278 taken from launder	1.8
G.	From pump in adit level close to surface	3.01 ¹

Samples *A*, *B* shewed minute, and *C* and *E* rather larger traces of copper. No arsenic was present in any of them.

To compare with these, the salt in samples from some of the inland mines was estimated. The results were :

					NaCl per cent.
East Pool	200	0.009
"	adit	0.018
South Crofty	Eastern engine	0.023
"	Western engine	0.013
Dolcoath	375 Harriet	0.090
Silverdale	no. 6 pit	0.006
"	no. 14 pit	0.004

We have previously (this volume, p. 86) shewn that less than 2 p.c. salt is sufficient to kill the larvae (especially when first hatched) of *Ankylostoma*, and I have since repeatedly confirmed this result. Such traces of copper as are present in the Levant water seem harmless to the larvae. It would appear then that this mine has escaped the disease from the fortunate circumstance that the roof was in times past left rather thinner than is altogether desirable.

The next table summarises these results and shews the distribution of 158 non-infected miners according to the percentage content of their blood in eosinophile leucocytes :

	Cases	<1	1—	2—	3—	4—	5—	6—	7—	8—	9—	10—	15—	20—	25—	> 30	Av. per- centage
III. (a) Talke o' th' Hill	40	13	10	8	5	2	1	1									1.9
(β) Snailbeach	42	7	18	7	5	1	1	2	1								2.1
(γ) Levant	41	5	9	12	6	4	1	1		1					1	1	3.7
(ε) Surface workers at Dolcoath	14	3	4	2	3	2											2.0
(γ, δ) Other underground workers	21	1	8	3	4	2	2	1									2.7
Total	158	29	49	32	23	11	5	5	1	1					1	1	2.5

¹ Atlantic sea-water contains 3.4 p.c. NaCl.

C. Partly Infected Mines.

Blood films were obtained from the men at two of the smaller tin-mines in the Camborne district. These mines are cool (62° to 68° or 70° F.) and not very wet, and, though there is a considerable exchange of men with Dolcoath, no cases of actual illness could be found. Indeed I saw several men who had been pale and short of breath while at Dolcoath and who had lost all their symptoms after coming to these mines: and this without any specific anthelmintic treatment. That this was not due to the mines being free from *Ankylostoma* was shewn by the discovery of the eggs in four out of 23 samples of faeces obtained at East Pool. These recoveries and the absence of illness are no doubt due to the unfavourable underground conditions which prevent the worm gaining a very firm footing; in this way repeated re-infections of the men are less frequent.

i. *East Pool and Agar United*. No illness. Twenty-three samples of faeces shewed, *Trichocephalus* 14, *Ascaris* 9, *Oxyuris* 1, *Ankylostoma* 4. Blood films obtained from 40 men (underground complement 223 = 18 p.c.) gave the following results.

These shew 14 cases under 5 p.c. and 17 over 10 p.c. of whom 3 were over 20 p.c. Careful inquiries were made of each man as to previous employment at Dolcoath: the four who had so worked are marked (*), and it is interesting to note that they all shew an eosinophilia of more than 10 p.c.

ii. *South Crofty*: a small cool tin-mine. No illness. No faeces obtainable as the mine had just been cleaned up. Twenty-two films obtained which gave the following results (underground complement 129 = 17 p.c.).

	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
T. S.	26.8	6.2	5.2	60.4	1.0	0.4
C. R.	29.4	11.0	3.8	52.8	1.4	1.6
W. H. M.	34.2	4.2	3.2	56.6	1.4	0.4
— S.	19.0	5.0	5.8	68.2	1.8	0.2
L. H.	26.6	4.8	2.0	64.2	2.2	0.2
W. H. J.	25.2	5.6	2.8	63.4	2.2	0.8
W. J.	31.0	6.4	4.2	55.0	2.8	0.6
— F.	26.0	5.0	5.8	59.2	3.2	0.8
W. S.	31.0	10.6	4.0	51.2	3.2	0 ¹
J. T.	18.6	3.0	4.2	70.8	3.4	0 ¹
E. L.	28.2	5.8	4.4	57.8	3.8	0 ¹
— W.	27.6	9.4	11.2	47.4	4.2	0.2
E. H.	25.4	3.8	3.8	62.4	4.2	0.4
E. R.	21.0	9.6	4.4	60.4	4.4	0.2

¹ Mast cells present.

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	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
J. H.	23·4	5·0	2·2	63·4	5·2	0·8
J. C.	34·8	3·6	3·4	51·0	5·6	1·6
H. L.	31·2	12·0	3·6	45·0	6·2	2·0
S. J.	24·8	7·8	7·2	53·4	6·2	0·6
A. S.	29·8	5·2	4·4	53·8	6·4	0·4
A. J.	19·0	4·6	4·4	64·8	6·4	0·8
—, W.	16·8	8·4	5·2	61·4	7·0	1·2
—, C.	24·0	5·2	4·0	57·4	8·4	1·0
—, L.	21·8	11·2	11·0	46·6	9·0	0·4
*J. W.	21·6	8·8	5·8	53·4	10·4	0 ²
—, W.	43·2	9·8	2·4	32·8	11·6	0·2
W. W.	24·2	6·0	2·2	56·0	11·6	0 ²
H. J.	15·0	4·6	3·0	65·0	11·8	0·6
C. V.	27·2	4·6	3·4	51·4	12·6	0·8
—, H.	26·8	4·4	5·8	49·0	13·4	0·6
W. L.	12·4	9·4	10·0	53·6	13·6	1·0
W. G.	20·8	5·0	3·2	56·6	13·8	0·6
—, J.	23·4	4·0	1·2	53·4	17·0	1·0
F. N.	23·8	4·0	2·2	52·4	17·2	0·4
F. G.	19·4	2·0	1·0	60·4	17·2	0 ²
*J. M.	31·2	5·2	3·2	40·6	18·4	1·4
—, K.	28·4	6·0	7·2	38·4	18·4	1·6
*J. C.	26·0	4·2	2·8	46·6	19·8	0·6
F. W.	8·2	4·4	3·4	61·8	21·8	0·4
W. G.	25·4	6·0	3·0	39·2	25·6	0·8
*W. W.	15·4	6·8	4·4	43·0	30·2	0·2
J. A. V.	35·2	7·6	5·4	50·4	0 ³	1·4
W. J. L.	41·6	15·6	3·8	38·2	0·2	0·6
W. L.	31·0	4·4	2·8	61·0	0·4	0·4
W. W.	12·4	8·2	2·8	75·6	1·0	0 ²
C. M.	26·4	6·2	2·2	63·2	1·8	0·2
W. P.	41·8	8·2	3·4	43·4	2·2	1·0
T. G.	24·4	6·2	6·8	60·0	2·2	0·4
W. G.	17·0	12·6	3·0	65·0	2·2	0·2
T. C.	24·8	2·8	2·2	66·4	3·0	0·8
W. R.	22·6	5·8	1·8	66·4	3·0	0·4
—, R.	33·0	5·8	3·8	53·2	3·6	0·6
J. H.	22·6	2·6	3·0	67·2	4·6	0 ²
L. F.	34·2	19·2	10·4	29·6	5·4	1·2
—, N.	27·4	8·8	4·0	53·4	5·6	0·8
W. B.	32·6	4·2	0·8	55·8	6·2	0·4
—, H.	32·8	10·6	5·2	43·2	6·8	1·4
G. D.	44·2	7·8	3·8	36·4	6·8	1·0
W. T. M.	23·8	9·4	5·2	54·0	7·2	0·4
J. C.	23·6	3·0	3·8	51·6	16·4	1·6
J. O.	25·2	4·6	3·0	47·6	18·6	1·0
P. O.	22·4	2·2	1·8	47·2	26·0	0·4
¹ T. W.	20·2	4·2	1·8	44·6	27·4	1·8

¹ Worked at Dolcoath 7 years since, when he was pale and dyspnoeic : soon recovered in this mine.

² Mast cells present.

³ Eosinophiles found.

The prominent feature of the series for these two mines is the large number of cases which shew a low degree of eosinophilia. Comparing the figures with those from the two other series we find that, while of the infected miners 2·7 p.c. fall between 5 and 8 p.c. and of the non-infected miners 7·0 p.c., at these mines no less than 21 p.c. have eosinophile counts within these limits. This is probably due to the frequent infection with *Ascaris* which is present; the results obtained at Levant shew that *Trichocephalus* may probably be excluded. It has unfortunately not been possible to investigate these cases individually; and it is useless to speculate in the matter. There is no doubt that the high figures, in most instances at any rate, indicate *Ankylostoma*.

The blood counts from these two mines are not taken into consideration in the following discussion.

If we contrast the figures of the Dolcoath cases with those obtained for the series of 158 non-infected miners, the result is very striking (the figures are given as percentages of the total cases):—

Percentages of Eosinophiles.

	No. of cases	<1	1—	2—	3—	4—	5—	6—	7—	8—	9—	10—	15—	20—	25—	>30	Av. percentage
A. Infected	148	0	0	0	2·7	0·7	1·3	0·7	0·7	4·7	6·1	27·0	23·0	14·9	5·4	12·7	18·2
B. Not infected	158	18·35	31·0	20·25	14·6	6·3	3·2	3·2	0·6	0·6	0	0	0	0·6	0·6	0	2·5

The same figures are illustrated graphically in the accompanying chart, p. 454.

If these figures are examined it will be seen that among the infected cases there is a sudden increase in frequency at 8 p.c., while in the non-infected cases the curve has at this point practically reached zero. It is impossible to lay down any hard and fast rule as to what absolute number of eosinophiles per cubic millimetre of blood should be regarded as pathological; still less therefore can one define the corresponding point in percentage figures. From general experience one is justified in regarding any figure under 5 p.c. as being normal, though something must be amiss if a series of cases gives an *average* of 5 p.c.; above 10 p.c. one may say definitely that there is an eosinophilia. Between 5 and 10 p.c. cases are much more doubtful. The figures brought forward here indicate clearly that anything above 8 p.c. is to be regarded with suspicion, and seem to justify our placing the upper limit of the normal at 7 or 8 p.c. This figure is also in accord with general experience,

though isolated cases of one sort and another have occurred in everyone's experience in which an eosinophilia of this degree has been explicable on no known ground. I would propose therefore, for the present practical

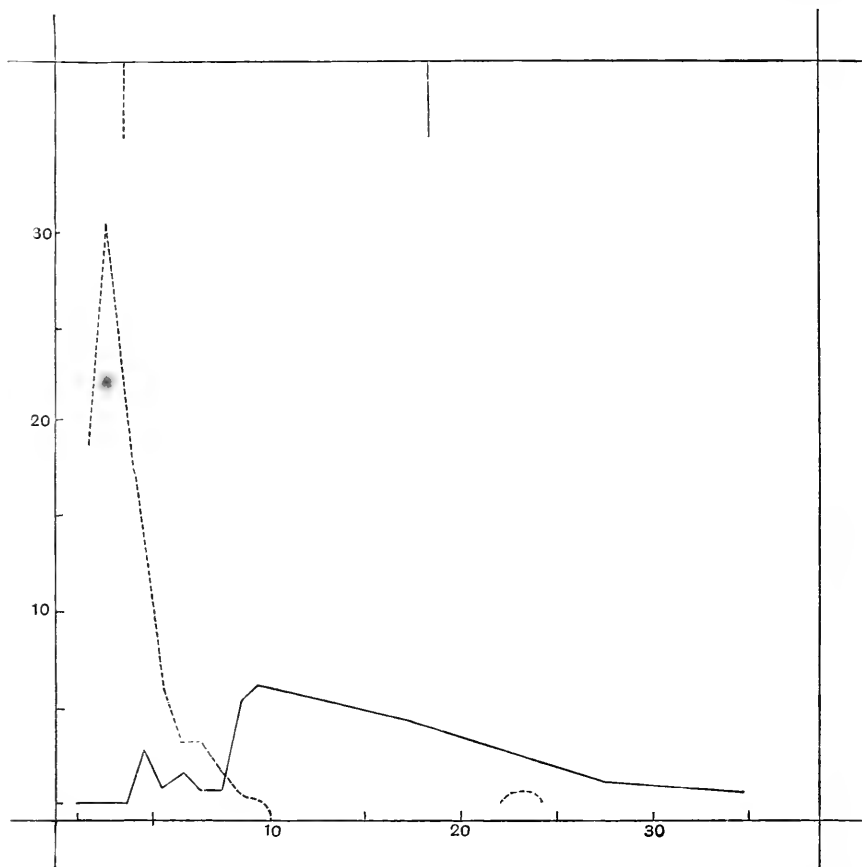


Fig. 1. The ordinates represent the percentage frequency of occurrence of each percentage of eosinophiles in the two series, the abscissae the percentages of eosinophiles. The continuous line represents infected, the interrupted line non-infected, miners.

purpose, to regard any figure under 5 p.c. as normal, numbers between 5 and 8 as doubtful, and any count of over 8 p.c. as extremely suggestive. On this basis the figures work out as follows:—

	No. of cases	under 5 p.c.	5—8 p.c.	over 8 p.c.	over 20 p.c.
Infected	148	3·4 p.c.	2·7	93·9	33·1
Not infected	158	91·1	7·0	1·9	1·3 p.c.

If we make allowance on the one hand for those doubtful cases which are reckoned as infected (group A ii. β and γ , p. 441), and on the other hand for the two cases of high eosinophilia among the "non-infected" group for which a definite cause (*Ascaris* infection) was found, the figures become even more emphatic. And it is clear that the frequency of an eosinophilia in those infected with *Ankylostoma*, and its rarity among other miners, renders the method of examination by means of dried films one of considerable value. Among all the procedures of what may be called laboratory diagnosis of disease, there is not one which is not liable to those exceptions which occur in any other symptom or physical sign in every series of cases: there is in short no infallible method known—be it a Widal's reaction or an examination of the sputum for tubercle bacilli or of the morphology of the blood¹—whereby we may put our penny in the slot and draw out a correct diagnosis every time². Every such examination must be made with a knowledge of what it means, and interpreted in the light of adequate information as to both the individual case which may be in question and also the occurrence of similar changes under other circumstances. It will be necessary therefore to consider (1) methods, (2) circumstances which influence the eosinophilia of *Ankylostoma*, and (3) the other conditions in which an eosinophilia occurs. It will then be possible to arrive at a truer estimate of the value of figures which have been obtained.

I. *Methods.*

We are here for the time only concerned with the methods of making a differential examination of the leucocytes in dried blood-films.

(1) *Method of making films.* Those used in this inquiry have been made in three ways:—(1) by spreading the drop between two cover-glasses, (2) by smearing a drop on a slide with cigarette paper, and (3) by smearing a drop on the slide with the end of another slide. The latter method is, for purposes such as the present, by far the best. The films have, for the most part, to be made when and where the subjects can be best approached; in the case of metalliferous mines there is usually a "dry" (changing-house) which is warm and very dusty. In

¹ Even in medullary leukaemia the blood may at intervals be quite normal. The author has seen such a case.

² We are I believe indebted to Dr Otto Grünbaum for this happy expression of a truth which is often forgotten.

coal-pits it is difficult to avoid taking the films underground; under circumstances such as these, with a very imperfect light and one's hands and belongings covered with mud and coal-dust, any method of using cover-glasses is out of the question. The combination of hurry and dirt requires something less fragile and I have found that the method of using two slides answers admirably. If films have to be taken underground it is well to avoid the face or the return air-ways: the air here will be so moist that the films take a very long time to dry. At the same time it is surprising what good specimens can be obtained even when a thin film is not dry for ten or fifteen minutes, and even then only when warmed over a safety-lamp: this slow drying results in an abundant coating of coal-dust, but the histological, if not the aesthetic, appearances in the stained film are all that are necessary, and often all that could be desired.

Slides have the further advantage that they are easier to clean than cover-glasses, and as the film is examined without mounting there is no trouble in using the same glass many times. I have found it convenient to carry the slides in ordinary envelopes, ten or a dozen in each. These can easily be carried about, and the slides when used are replaced in them. There is no need to keep the films apart; once dried they may safely remain in contact with one another. They must however be kept quite dry. Before visiting the mine, etc., each slide should have a small label affixed in which the subject's name etc. may be written when the film is taken.

Apart from considerations of practical convenience it is not clear that it is a matter of indifference which method is used. The cover-slip method gives probably the best results and is open to less theoretical objection than any process in which the blood is dragged along a slide. The method of cigarette paper seems to be thoroughly bad; a large number of the leucocytes are entangled in the paper, with the result that the differential count takes longer to make and one is unable to judge of the presence of a leucocytosis by looking at the stained film alone. Moreover it may be that the different varieties of cells are mopped up by the paper equally; this does not appear to be the case. It would seem that the neutrophile and eosinophile cells are taken up proportionately more than the others. The following figures shew the results of differential counts (500 cells) in two cases where simultaneous preparations were made by smearing the blood with cigarette paper and with another slide:

	Mononuclear cells	Neutrophiles	Eosinophiles
A. Cigarette paper	42.0	53.2	4.4
Slide smear	32.6	60.8	5.4
B. Cigarette paper	50.0	44.0	5.0
Slide smear	37.8	52.6	7.6

The same objections hold, in the case of smearing with two slides, but I believe in a very minor degree. The larger cells must necessarily be to some extent separated from the smaller in this method if one attempts to make very thin films. Such films are however not advisable for the purpose of differential counts; not only are the white cells scattered over an area inconveniently large but many are destroyed. There is little doubt that the appearances in exceedingly thin films are less reliable than in somewhat more thickly spread preparations. In using two slides the thickness of the film can be nicely regulated at will, and if not too thin the differential count is probably as accurate by this as by any other method except those which make use of fresh wet preparations.

(2) *Staining.* For all practical purposes, no stain is at once so good and so convenient as a solution of eosinate of methylene blue in methyl alcohol (Jenner's stain). For the present series, a $\frac{3}{4}$ p.c. solution of Grübler's dry stain in Merck's methylalcohol has been used, staining for $2\frac{1}{2}$ minutes in a tube of stain; this may be used (with the occasional addition of a few drops of fresh solution) for at least 200 slides.

(3) *Examination.* Good films, well-stained, may after some experience be adequately examined with a $\frac{1}{3}$ inch or lower power, and the presence or absence of an excess of eosinophiles is seen at once. If a higher power is necessary, it is convenient to use an immersion, since it is then not necessary to mount the film. If an actual numerical estimation is wanted, a count of 500 is sufficient for practically all purposes; to count more than this is to strive after a precision which the method cannot possess. We have previously pointed out¹ that there is an error in the differential count of definite magnitude. The following figures shew the percentages of neutrophiles and eosinophiles in successive five hundreds on the same film in two cases:—

	A		B	
	Neutrophiles	Eosinophiles	Neutrophiles	Eosinophiles
1st 500	54.4	9.0	76.4	2.2
2nd 500	53.6	10.4	72.2	2.6
3rd 500	53.4	11.4	74.4	1.0
4th 500	50.0	10.8	76.2	1.2

¹ This *Journal*, III. 1903, p. 115.

In both these cases care was taken that the whole 2,000 should be counted on as uniform a part of the film as possible. If the extreme margins of any (and especially smeared) films are counted, extraordinary and obviously unreliable figures are often obtained.

If no greater degree of accuracy than these figures shew is to be obtained by counting 500, it is interesting to inquire whether 200 would be sufficient. Except for those cells which occur in small numbers (eosinophiles and mast cells in normal blood), the results obtained from these shorter counts are fairly accurate:—

		A	B	C	D	E	F
200	{neutrophiles	45.0	68.0	37.0	44.5	52.5	58.0 p.c.
counted	{eosinophiles	11.5	17.0	8.0	21.5	8.0	14.5 „
500	{neutrophiles	46.2	66.2	41.6	45.6	55.6	58.0 p.c.
counted	{eosinophiles	15.0	18.6	8.2	19.8	9.8	12.0 „

II. *The Eosinophilia of Ankylostoma infection and ankylostomiasis and the conditions which influence it.*

(1) *Influence of the stage of the disease.* Previous observers have dealt almost entirely with the blood in those who are definitely anaemic, *i.e.* with the blood in ankylostomiasis. As far as I am aware no previous series of blood examinations of cases of *Ankylostoma* infection in persons who shew no symptoms of disease has been published. The phenomena exhibited by the leucocytes are not necessarily the same in the two classes. In our first paper¹ it was pointed out that the eosinophilia was most marked in cases in young persons which were of comparatively recent date and which did not necessarily shew any very severe diminution of the percentage oxygen capacity of the blood. Of such cases which we were fortunate enough to examine in the stage at which they shewed a distinct and high eosinophile leucocytosis comparable to that found in trichinosis, the following are good examples:—

	Age	History	Hb p.c.	Leucocytes per c.mm.	Eosinophiles	
					per cent.	per c.mm.
S. C.	23	1 month	80	56,000	66.2	37,000
E. J. T.	18	6 months	98	20,600	56.2	11,500

It was also pointed out that the numbers of leucocytes corresponded in a general way to the percentage of eosinophiles, though this phenomenon was not well marked until a definite leucocytosis of some 13,000 was reached. When however a stage of definite anaemia is

¹ This *Journal*, III. 1903, p. 121.

reached, such leucocytoses seem to be far less frequent, and in many cases the leucocytes per unit volume are distinctly less than normal. Leucocytoses do however undoubtedly occur, and it would seem mostly in those who have reached a condition of anaemia very quickly. Thus both the following cases had only short histories of dyspnoea, etc.:—

	Age	History	Hb p.c.	Leucocytes per c.mm.	Eosinophiles	
					per cent.	per c.mm.
J. C.	17	6 months	50	44,000	72.7	32,000
N. S.	17	2 months	40	24,400	29.0	7,000

Of those who have reached a profound grade of anaemia many shew a diminution of the total leucocytes. B. K. Ashford and W. W. King¹ have recently described a number of such cases in a valuable series, in which counts of 2,000 to 4,000 were frequently found in cases which were extremely anaemic². This diminution may be due to two factors: (1) *The dilution of the blood*: we have already shewn that this is the main cause of the diminution of the percentage haemoglobin content of the blood (vol. III. p. 112), and it is clear that the leucocytes per unit volume might be affected in a similar way. But there are at least two reasons for thinking that this factor cannot play any great part in concealing a leucocytosis. In the first place, leucocytoses may occur with marked anaemia. And, secondly, the blood always shews a strong tendency to restore its volume and percentage composition to the normal when any deviation is brought about by experiment or disease. A comparable example of this tendency is found in the normal leucocytic content of the hydraemic blood of chlorosis; though the blood is diluted some two or three times the leucocytes per unit volume remain about normal, and during cure the concentration of the blood may produce the appearance of a definite leucocytosis. Here the volume of the blood is altered beyond immediate cure, so that the efforts of the haemopoietic system can only reach the normal in so far as the percentage composition is concerned. From such considerations as these it does not seem

¹ *American Medicine*, vi. 1903, p. 391: some doubt is here thrown upon the accuracy of our observations relative to the existence of high eosinophile leucocytosis due to *Ankylostoma* because the authors have not been fortunate enough to meet with such cases. Ashford has however himself recorded a good example (7,200 eosinophiles in 18,000 total leucocytes), though he prefers to consider that it was due to a pneumonia of a somewhat hypothetical nature.

² The very low figures for the haemoglobin (7—12 p.c.) given here must be regarded with suspicion in the absence of any evidence as to the accuracy of the standardisation of the instrument used. I have recently found that in this part of the scale the Miescher-Fleischl apparatus gives results about 25 to 30 p.c. too low.

probable that the hydraemia of ankylostomiasis would produce an apparent diminution in the leucocytes, but rather that the leucogenic tissues would proliferate to the degree necessary to maintain the number per unit volume at the normal or abnormal figure produced by the presence of the morbid agents. (2) The diminution of the total leucocytes may in the second place be due either to a cessation of the necessity for the reaction, or to a failure on the part of the marrow to produce it. There is little doubt that in time many individuals become practically immune to such worms as they may harbour. Several instances have been noted in Cornwall in which severe attacks of anaemia have passed off, leaving the miner with a normal Hb content and yet with abundant eggs in his stool. These cases especially occur where, by change of occupation, repeated reinfections are avoided, though they have also been found in those who have constantly been exposed to infection. Some of these shew low counts of both total leucocytes and of eosinophiles: on the other hand several shew high degrees of eosinophilia but no definite leucocytosis. But it would seem that the capacity of the marrow to keep up the eosinophilia may fail in those who are greatly reduced by long-standing anaemia or by inter-current disease. Though we cannot deny that such diminution may be in part due to the dilution of the blood, yet this cannot be the whole explanation since the percentage of eosinophiles in many of these cases is also diminished¹. Such results find a reasonable explanation in the partial exhaustion of that part of the leucogenic apparatus which has been chiefly stimulated, and the failure on the part of the individual to react, as shewn by a low percentage of eosinophiles, is with much justice regarded by Ashford as of unfavourable prognosis².

Ashford and King record 86 differential counts in 62 cases, all of whom were severely anaemic when first seen. Grouped in a similar way to our own figures, their results are given in the following table, together with 19 others previously recorded by Ashford³.

	Counts	< 5 p.c.	5-8 p.c.	> 8 p.c.	> 20 p.c.
Hb under 50 p.c.	82	17 = 21 p.c.	15 = 18 p.c.	50 = 61 p.c.	11 = 13 p.c.
Hb over 50 p.c.	23	1 = 4	3 = 13	19 = 83	9 = 39
Total	105	18 = 17	18 = 17	69 = 66	20 = 19
Cornish cases	148	3.4 p.c.	2.7 p.c.	93.9 p.c.	33.1 p.c.

¹ Our own figures do not shew this clearly, but the number of cases of severe anaemia seen in Cornwall has been comparatively very small.

² *American Medicine*, vi. 1903, p. 391.

³ R. C. Cabot, *Clinical Examination of the Blood*, ed. 4, 1903, p. 433.

These results shew a frequency and intensity of eosinophilia considerably less than that found in our series. There are three factors which may be considered: (1) the degree of anaemia in the two series is altogether different: some 20 only of our Cornish cases had less than 50 p.c. Hb. That this is the chief influence at work is rendered probable by the figures found by Ashford in cases which were to some extent cured; these correspond much more closely to our own results. But at the same time these improved cases are not necessarily comparable to ours, who were, except in a few instances, untreated men who had never suffered from any considerable anaemia, and in whom the worm was present in considerable numbers. (2) The people concerned are different in race, previous history, etc. It is impossible to assess the influence of this factor. They have doubtless in many instances harboured other intestinal parasites, so that it might be thought that their power of or need for an eosinophile reaction would have been thereby diminished. The Cornish miners, however, are infested with other worms to a degree which seems to be at least as great as that found in the tropics (see p. 449). (3) The worm concerned is different. All Ashford's cases are from Puerto Rico and are due to *Ankylostoma americanum*, Stiles. Until the factors of duration and degree of affection can be excluded we can only suggest that this may influence the intensity of the eosinophilia. The numerous cases scattered through recent American literature all apply to cases of anaemia and shew generally the same figures as those recorded by Ashford. It is, however, somewhat suggestive that the figures in those cases due to *A. duodenale* (of which a few have been imported to the United States) tend to be rather higher than those due to the other species. The cases are however too few to give any definite conclusion. It is very desirable that a study should be made of cases of infection by *A. americanum* apart from ankylostomiasis.

The conclusion may however be drawn that the eosinophile reaction is most constant and best marked in those who are not suffering from definite anaemia, that is, in just those persons who shew nothing which would suggest that they harbour the worm. For the purposes of practical hygiene this is a most fortunate occurrence.

(2) *Onset of eosinophilia after infection.* The period which must elapse between infection and the appearance of the increase in eosinophiles is necessarily very difficult to determine apart from direct experiment. The following circumstances occurring in a laboratory worker were however very suggestive, and in view of the lack of definite information seem to be worth recording.

	Date	Hb p.c.	Total leuco- cytes per c.mm.	Lympho- cytes	Inter- medialtes	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
Nov. 10. First contact with any <i>Ankylostoma</i> material for a period of 11 months: during that time blood and stools had been repeatedly examined with completely negative results. On this date some infected faeces received from Cornwall.	30. x. 03	96	8050	26.9	6.2	5.0	59.4	3.0	0.4
	11. xii. 03	97	7800	17.6	15.0	2.8	53.6	10.4	0.6
	18. xii. 03	95	7000	19.8	14.8	2.4	52.8	8.2	2.0
	2. i. 04	98	4500	29.4	10.8	4.2	47.8	6.8	1.0
Nov. 21. First contact with encapsuled larvae which had been bred in the laboratory at a low temperature. Blood not examined till Dec. 11. Stools repeatedly examined with negative results till Jan. 28, 1904, when a few eggs were found; they were found again on Feb. 3, but were always very few in number and many negative examinations were made.	4. i. 04	98	7400	19.6	17.6	1.8	51.4	9.0	0.6
	1. ii. 04	94	9000	31.4	8.2	1.6	49.8	7.8	1.2
	12. ii. 04	101	7400	31.8	14.4	3.8	44.0	5.0	1.0
	8. v. 04	100	7900	16.4	10.6	6.6	59.2	7.0	0.2
On May 17, owing to an accident with a pipette, it was thought possible, though not likely, that some larvae had been swallowed. The blood was frequently examined during the next fortnight, but no material change was found. No eggs could be found on repeated searches. No examination of the blood was thereafter made for three weeks, when a well-marked eosinophile leucocytosis was found. Faeces searched regularly for eggs, but none were found till July 11; after this eggs were present in small numbers every day, though they could only be found with the greatest difficulty.	13. v. 04	—	—	30.2	11.2	1.6	49.4	7.4	0.2
	17. v. 04	101	7970	21.2	14.8	0.4	59.2	3.4	1.0
	31. v. 04	—	—	30.0	7.6	2.2	48.6	10.0	1.6
	22. vi. 04	97	15000	13.4	9.6	1.2	50.8	24.0	1.0
	27. vi. 04	103	9170	22.0	8.4	2.4	32.8	33.6	0.8
	4. vii. 04	104	13200	23.0	5.0	1.4	30.2	40.4	0

A definite eosinophilia was thus found 20 days after the first possible contact with encapsuled larvae. No large amount of work with this material was done until Nov. 28 and the following days. No skin eruption was at any time present. As far as is definitely known the worms do not reach maturity for at least 30 days after infection, and in skin infection Looss has found an interval more than twice as long elapse before eggs could be found. In this case eggs were found 68 days after the first possible infection and 48 days after the eosinophilia was noted. Later on there was evidently a reinfection; eggs were found 55 days after the date on which this probably took place. Unfortunately the blood was not examined during a critical period of three weeks, but there was a definite rise in the eosinophiles 14 days after the probable infection, and 19 days before eggs could be found: a considerable eosinophilia with a moderate leucocytosis was present. The case also illustrates how the blood change may be present in a very marked degree when eggs are present in such small numbers that they would often be missed by the ordinary method of examination. During June and July some pain was experienced of a dyspeptic character but without any relation to the taking of food: there have been no other symptoms¹.

(3) *Relation of eosinophilia to treatment.* The effect of efficient treatment is uncertain and inconstant. The eosinophiles never seem to diminish quickly, and in many cases shew at first a rise. The following figures are from two cases which have been treated till no eggs were to be found in their stools.

	Date	Hb p.c.	Differential counts					Mast cells	
			Lympho-cytes	Inter-mediate	Large hyalines	Neutrophiles	Eosinophiles		
J. S. 51	31. x. 02	48	13·4	7·4	6·2	56·2	16·0	0·8	Treated several times during 6 months
	15. xi. 02	43	10·8	5·6	3·4	61·0	18·0	1·2	
	vii. 03	64							
	3. xii. 03	92	13·4	14·4	1·4	55·4	14·8	0·6	No eggs found
	7. xii. 03	—	14·0	18·4	1·2	55·6	9·8	1·0	No eggs
	8. ii. 04	—	35·4	4·4	1·4	44·4	13·4	1·0	No eggs
R. C. 30	5. ii. 04	49	26·6	4·4	5·8	49·2	13·8	0·2	Treated 7. ii. 04
	20. ii. 04	58	16·0	7·6	2·8	62·4	9·8	1·4	
	22. iii. 04	72	31·0	9·0	2·4	55·6	2·0	0·0	No eggs
	29. iv. 04	90	42·2	6·0	1·6	37·0	12·6	0·6	

¹ The opportunity has been taken of shewing in this case that the fluid in blisters from burns and in the various stages of superficial skin infections contains no excess of eosinophiles though in the blood this variety was more numerous than the neutrophiles.

The second case fell at one time to a normal figure, but afterwards shewed a recrudescence of the eosinophilia. The patient in this case had had symptoms for about 6 months only, while in the first case they had been present in a severe degree for some 4 years. We have not observed a case where the eosinophilia disappeared after treatment, and in many cases it undoubtedly remains for a considerable time. We have examined four men who had given up underground work some time previously on account of their anaemia, etc., and in whose stools no eggs could be found. They shewed an eosinophilia varying from 11 to 22 p.c., and are to be regarded as cases in which a natural cure has taken place after removal from any chance of reinfection, leaving however a marked eosinophilia.

Ashford and King have had the opportunity of following a number of cases till the worms were all expelled and the blood restored to an approximately normal condition. With regard to the eosinophilia they conclude that "there is a final return to normal after the patient is cured¹." The figures given to illustrate this are however similar to those which we have obtained and by no means bear out the authors' conclusion :

Case no.	Before		After	
	p.c. Hb	p.c. eosin's	p.c. Hb	p.c. eosin's
VIII	45	12.5	70	7.5
LXV	22	12	75 circa	5
LXX	21	15	94	15

No definite conclusions as to the presence of worms after treatment can therefore be drawn from the degree of eosinophilia which remains, and this persistence must be particularly considered when using the blood-film method of examination. This method is I think practically valueless where a mixed lot of treated and untreated men are under examination. It is to be suspected that the degree and duration of the residuary eosinophilia would be greater in those who have been infected for a long period, but we have no figures which definitely shew this².

(4) *Influence of intercurrent disease, etc.* Intercurrent diseases which themselves produce more or less characteristic changes in the blood may influence the differential count either by concealing per-

¹ *loc. cit.* p. 394.

² A doubt was previously (this *Journal*, III. p. 127) expressed as to the validity of the evidence adduced by Ehrlich in support of his statement that an eosinophilia persists for a long time after expulsion of the worms. His statement is, it appears, correct, though the case cited by him does not shew it.

centage eosinophilia by a large increase in the number of other varieties, or the diminution may be absolute and due to the presence of substances which are negatively chemiotactic to the eosinophiles. In many instances both of these factors would be at work. A normal absolute eosinophile count can on the contrary be raised to a percentage eosinophilia by a considerable diminution in the total leucocytes¹. Our information on these points is very deficient. Leichtenstern² observed that the incidence of a lobar pneumonia reduced the percentage from 72 to 6, the figures rising again to 54 p.c. on recovery from the pneumonia. In the case of a Dolcoath miner (*Ankylostoma* eggs found, 45 p.c. Hb) we found in the film an obvious large leucocytosis with 77.4 p.c. neutrophiles and only 3.8 p.c. eosinophiles; ten days later the leucocytosis was much diminished and the neutrophiles were only 55 p.c., while the eosinophiles had risen to 15 p.c. The eosinophilia of his *Ankylostoma* infection was obscured at the time of the first examination by a neutrophile leucocytosis of undetermined origin³.

It is somewhat remarkable that hardly any cases of these disturbances of the eosinophile count have been noted in the long series of blood examinations which have been made in infected miners, and which have included men suffering from pleurisy, miners' phthisis, tubercular peritonitis, carcinoma of stomach, etc. Factors such as time of day, period of digestion, etc., produce such small changes that for practical purposes they may be neglected. Far too much stress has been laid on the necessity of avoiding *e.g.* digestion leucocytosis in making differential counts. Not only is such leucocytosis very often absent in healthy people eating ordinary meals, but its magnitude is such that the error introduced is less than that inherent in the method. Practically, it is necessary to make a careful note of any abnormal conditions present⁴ when taking the blood film, and if necessary make allowances for them. If an eosinophilia is obscured by a neutrophile leucocytosis, the abundance of leucocytes and the high percentage of neutrophiles will readily suggest the real state of affairs.

¹ This is actually seen in some anaemias.

² P. Ehrlich and A. Lazarus, *Die Anaemie*, Part 1. p. 113.

³ The most probable cause seems to be a latent carcinoma; the patient (aet. 51) has symptoms of gastric trouble.

⁴ It is well to remember that trivial boils may give rise to a very considerable inflammatory leucocytosis.

III. *States which are accompanied by an increase in the eosinophile leucocytes of the circulating blood.*

Since the rise of haematology a large number of conditions have been described in which an increase of eosinophiles has been observed. Such increase may be relative only to the number previously present, as for instance during the subsidence of a pneumonia. It is however proposed to summarize here only those conditions which cause an increase relative to the average normal quantity, and of such an order that about 10 p.c. at least are found. It is desirable that this should be done in order that we may be in a position to use the blood-film method of examination for *Ankylostoma* to the best advantage and interpret the results obtained in a proper manner. The extensive literature on the subject is in many parts rendered of little value for our present purpose owing to two circumstances. In the first place, an unfortunate prominence has always been given to positive cases: there are indeed very few conditions in which the frequency of occurrence of an eosinophilia has been worked out. It follows that in several abnormal states eosinophilia is commonly thought to be more frequently present than it really is. In the second place, especially in the older literature, adequate attention has not been paid to the possibility of the sporadic presence, among a series of cases of some disease, of certain individuals who are affected with some condition which, while giving rise to no obvious symptoms, may cause an eosinophilia. The condition above all others which thus requires to be constantly considered is the presence of intestinal worms.

The conditions, which are associated with an eosinophilia, may be grouped under four heads¹:

(I) *Paroxysmal asthma:*

In true paroxysmal asthma a high eosinophilia (30—60 p.c.) has been frequently recorded², and some degree has been found in the asthmatical attacks occurring in emphysema. In true asthma some increase is said to be constant about the time of the paroxysms. I have examined a number of cases with negative results but in no instance could a secondary (pulmonary) origin for the asthma be strictly excluded.

¹ References to a few typical records are alone given. Many other references will be found in the text-books of Cabot, da Costa, Ewing, and Bezançon and Labbé, and in the memoirs referred to.

² J. S. Billings, *New York Med. Journ.*, vol. LXV. 1897, p. 692: H. S. French, *Practitioner*, LXX. 1903, p. 307.

(II) *Skin diseases* :

In pemphigus¹ and dermatitis herpetiformis², some degree (generally 10 to 20 p.c.) of eosinophilia is very frequent, but not constant. In other affections of the skin (chronic eczema³, psoriasis¹, lupus¹, urticaria⁴, etc.) high counts have from time to time been recorded. But it is exceptional to find an eosinophilia among any ordinary body of patients suffering from skin diseases. This is clearly shewn by the recent records of H. S. French⁵: of 90 cases, including most of the ordinary conditions, 13 shewed more than 5 p.c., and only 4 more than 10 p.c. I have examined a series of 11 cases of acute wide-spread urticaria: in no case did the eosinophiles reach 4 p.c.

(III) *Parasites* :A. *Trematodes*.

(1) *Bilharzia haematobia*. Eosinophilia is very frequent and often of a high grade: A. C. Coles⁶ 20 p.c., A. E. Russell⁷ 23·8 to 33·6 p.c. I have found more than 20 p.c. at some period in the 5 cases which I have examined, and no clear case of active disease with normal eosinophiles seems to have been recorded. In one case a count of 47 p.c. fell to 5 p.c. when the patient finally ceased to pass any ova or blood⁸.

B. *Nematodes*.

(2) *Ascaris lumbricoides*. High counts are sometimes met with (Solley 33 p.c.⁹): I have already mentioned (p. 449) two cases in adults with 23·8 and 25·6 p.c. But as a rule no marked increase is observed, and completely negative cases are recorded by Bücklers¹⁰, Boycott⁵, H. Limasset¹¹, etc.

(3) *Oxyuris vermicularis*. Bücklers has recorded a count of 16 p.c. in an adult: Limasset had four negative cases. I have examined

¹ J. Zappert, *Zeitschr. f. klin. Med.* xxiii. 1893, pp. 272—273.

² Laredde, *Annal. de Dermatol.* [3] ix. 1898, p. 1016.

³ P. Canon, *Deutsche med. Wochenschr.* xviii. 1892, p. 206.

⁴ Ehrlich and Lazarus, *Die Anaemie*, Part I. Transl. by Myers, p. 150.

⁵ *Guy's Hospital Reports*, 1903. It is very desirable that similar statistical records should be prepared for the other conditions in which eosinophilia occurs. Our information rests too much on the records of individual cases.

⁶ *Brit. Med. Journ.* 1902, i. p. 1137.

⁷ *Lancet*, 1902, ii. p. 1540.

⁸ *Brit. Med. Journ.* 1903, ii. p. 1268.

⁹ J. Ewing, *Clinical Pathology of the Blood*, ed. 2, 1904, p. 473.

¹⁰ *Münch. med. Wochenschr.* 1894, p. 22.

¹¹ *Thèse de Paris*, 1901, p. 78: the whole subject of the occurrence of eosinophilia in worm infections is here reviewed.

a series of 18 infected children: these shewed two with more than 10 p.c. (12·8 and 13·7), six between 5 and 10, and 10 with less than 5 p.c.¹ The conclusion is that while low grades are not infrequent, it is rare to find a high eosinophilia and quite common to find none.

(4) *Trichina spiralis*. Since the original discovery of the blood changes in this disease by T. R. Brown², a large number of cases have been recorded which shew the most extreme grades of eosinophilia; most of them afford good examples of a real eosinophile leucocytosis. The 21 cases collected by Cabot³ average (first examinations) 16,000 (8,000 to 28,000) leucocytes with 32 p.c. (10 to 67 p.c.) eosinophiles, and a high degree is almost constant. A single negative case (0·5 p.c.) has however been seen by da Costa⁴, and a probably negative one by Howard⁵. E. L. Opie⁶ has recently contributed a valuable study of experimental trichinosis in guinea-pigs, in which the production of a haemal eosinophilia and of local accumulations which practically amount to eosinophile abscesses is well shewn.

(5) *Filaria*. The eosinophiles vary from 4 p.c.⁷, through a number of cases shewing 7 to 20 p.c.⁸, to an extreme figure of 70 p.c.⁹ In *F. medinensis* A. Powell¹⁰ records six cases from 4·75 to 12 p.c., average 7·6 p.c. I am not aware that any series of cases has been published from which the frequency and grade may be properly ascertained, but it seems that most cases run at about 7 to 12 p.c.

(6) *Trichocephalus dispar*. No clear case of any marked degree of eosinophilia has been recorded. P. K. Brown¹¹ finds an eosinophilia a "strikingly constant symptom" in every case of worm infection, and says "in no less than ten or twelve cases where the *Trichocephalus hominis* alone appeared, the percentage of eosinophiles rarely fell below 5." My own experience leads to the conclusion that it is very exceptionally, if ever, that the presence of this worm gives rise to any

¹ *Brit. Med. Journ.* 1903, II, p. 1268. ² *Journ. Experimental Med.* 1898, p. 315.

³ *Clinical Examination of the Blood*, ed. 4, 1903, pp. 434 ff.

⁴ *Clinical Haematology*, 1902, p. 435: a local eosinophilia was found in the affected muscles.

⁵ *Phil. Med. Journ.* IV. 1899, p. 1085.

⁶ *Amer. Journ. Med. Sci.* CXXVII. 1904, p. 477.

⁷ E. Bloch, *Deutsche med. Wochenschr.* XXIX. 1903, p. 511.

⁸ A. C. Coles, *Brit. Med. Journ.* 1902, I, p. 1137; G. L. Gulland, *ibid.* p. 831; Vaquez, *C. R. de la Soc. de Biol.* LIV. 1902, p. 1425; J. A. Sicard, *ibid.* p. 1427; Calvert, *Johns Hopkins Hosp. Bull.* XIII. 1902, p. 133.

⁹ Rembinger, *C. R. de la Soc. de Biol.* LIV. 1902, p. 1145.

¹⁰ *Brit. Med. Journ.* 1904, I, p. 73.

¹¹ *Boston Med. and Surg. Journ.* CXLVIII, 1903, p. 585.

eosinophilia. At Levant Mine (see p. 447) *Trichocephalus* was found in 96 p.c. of the samples of faeces examined, so that it may be assumed that almost every man was infected. Yet of 41 blood counts only 5 shewed 5 p.c. or more, and the only two of these which were above 10 p.c. were satisfactorily accounted for by the presence of *Ascaris*. In the remaining three cases (5.0, 6.4, 8.4 p.c.) the blood condition may have been due to *Trichocephalus*; on the other hand comparable counts (5.2, 6.0, 6.8, 7.2 p.c.) were obtained at Snailbeach (p. 445) where no evidence of the presence of any worms could be found. E. Becker¹ found 2 p.c. in a case of severe anaemia (Hb 35) which he attributed to the presence of *Trichocephalus*.

(7) *Anguillula stercoralis*. I can only find four blood examinations in pure *Anguillula* infections: Bücklers² found 13.5 p.c., P. K. Brown³ 6.3 p.c., Pappenheim⁴ 0.8 p.c., and R. P. Strong⁵ 0.1 to 0.3 p.c.

(8) *Ankylostoma duodenale* and *A. americanum*. If we group together the Cornish cases (p. 460) with those recorded by Ashford (p. 460) we have in all 253, of which 82 p.c. shew over 8 p.c. of eosinophiles, 73 p.c. more than 10 p.c., and 27 p.c. more than 20 p.c.

C. Cestodes.

(9) *Taenia solium* and *T. mediocanellata*. Positive examples have been recorded by Bücklers (10.25 p.c.), Leichtenstern⁶ (34 p.c.), and others. H. Limasset⁷ counted 16 cases: five shewed at some period more than 5 p.c., but of these two only reached more than 10 p.c. (10.8 and 26.1). In Bücklers' six cases one was less than 5 p.c. and one more than 10 p.c. I have examined 8 cases: four were less than 5 p.c., three between 5 and 10 p.c., and one over 10 p.c. (13.0 p.c.). It would seem therefore that an eosinophilia is not common, and if present is usually of a low grade.

(10) *Echinococcus cysts*⁸. A high degree may occur: Achard and Clerc⁹ found 40 p.c., C. S. Seligman and L. S. Dudgeon¹⁰ 57 p.c., E. Bloch¹¹ 14.7 p.c. and Dargein¹² 12 p.c. On the other hand the reaction

¹ *Deutsche med. Wochenschr.* 1902, p. 468.

² *Münch. med. Wochenschr.* 1894, p. 22.

³ *Boston Med. and Surg. Journ.* cxlviii. 1903, p. 583.

⁴ *Centralbl. für Bakteriolog.* xxvi. 1899, p. 608.

⁵ *Johns Hopkins Hosp. Reports*, x. 1902, p. 94.

⁶ Ehrlich and Lazarus, *Die Anaemie*, Part i. Eng. Transl. 1900, p. 151.

⁷ *Thèse de Paris*, 1901, p. 66 ff.

⁸ Most of the cases are hydatids of liver.

⁹ F. Bezançon and M. Labbé, *Traité d'Hématologie*, 1904, p. 623.

¹⁰ *Lancet*, June 21, 1902.

¹¹ *Deutsche med. Wochenschr.* xxix. 1903, p. 511.

¹² *C. R. de la Soc. de Biol.* 1901, p. 969.

often fails: H. Limasset (5, 4, 0.6 p.c.), Bezançon and Labbé (5, 4 p.c.).

(11) *Cysticercus cysts*. H. Limasset records 10 p.c., C. H. Achard and M. Loeper¹ 11 p.c., and I have seen a case of generalised infection of two years' duration which shewed 8 p.c.; P. Marie² (2 p.c.) and others have recorded negative cases.

(12) *Bothriocephalus latus*. The information available as to the leucocytes in infections with this important parasite is very scanty, and all the records apply to anaemic cases. Schaumann, in his classical monograph, found a decrease in the eosinophiles, while the elaborate studies of E. Rosenquist³ include no mention of the matter. In J. Courmont's⁴ case the eosinophiles once rose to 7 p.c. during recovery but were otherwise normal. It appears from the records of Courmont, J. Drivon⁵ and others that *Bothriocephalus* is so common in the South of France, at Geneva (10 p.c.), and at St Petersburg (15 to 32 p.c. of autopsies) that, if any marked eosinophilia resulted from its presence, the fact would probably have been recorded. As far as our information goes, we must conclude that the presence of this worm does not cause an eosinophilia, but it is very desirable that the condition of the blood should be investigated in those who harbour the worm without suffering any ill effects from its presence. The profound anaemia which ensues in a few of the hosts may obscure the true leucocytic reaction to the parasite.

D. Miscellaneous parasites.

Pulex penetrans. F. Bushnell⁶ found 8.2 to 19.3 p.c. in a case of malaria; this was apparently due to the presence of a "jigger" in the foot.

Balantidium coli. E. Ehrnrooth⁷ found no eosinophilia in a case of chronic enteritis due to this parasite.

The presence of *Acarus*, *Pediculus*, the malarial parasite, *Trypanosoma*, the *Piroplasma* of the tick fever of Montana, etc. do not appear to give rise to any increase of eosinophiles, nor is any bacterial infection known which causes a similar blood change.

¹ Bull. de la Soc. Méd. des Hôpitaux (3), xvii. 1900, p. 867.

² Ibid. (3), xviii. 1901, p. 1126.

³ Zeitschr. f. klin. Med. xlix. 1903, p. 193.

⁴ Journ. de Physiol. et Path. Gén. v. 1903, p. 353.

⁵ Lyon Médical, May 4, 1902.

⁶ Clinical Journal, xxiii. 1903, p. 63.

⁷ Zeitschr. f. klin. Med. xlix. 1903, p. 321.

(IV) *Miscellaneous and indeterminate conditions.*

Sporadic cases are within the experience of everyone, and are scattered throughout the literature, in which the eosinophiles have reached the upper limits of what may be considered to be normal or have been definitely increased to moderately or very high figures in the persons of those who present no morbid condition which can be said to account for the state of their blood. In other diseases some authors have so constantly found a figure at about 5 or 6 p.c. that there would seem to be necessarily some connection of causative value. In this way increased figures have been recorded with certain malignant tumors, osteomalacia, gonorrhoea, gout, syphilis, etc., and a more uniform (though small) increase has been associated with scarlet fever, chorea, general paralysis, etc. High figures have commonly been ascribed to the blood of children, and E. Horder¹ has recently described a remarkable eosinophilia of high grade (about 20 p.c.) which is present in all the native population at Pakhoi in South China. Some stress has been laid on the frequency of such cases in minimising the diagnostic value of an eosinophilia²; yet it is extremely exceptional in ordinary blood examinations in this country to meet with these cases. It may be stated quite definitely that malignant growths, gonorrhoea, etc. do not as a rule cause an eosinophilia, though in a few individuals, whose blood has been examined because they were suffering from these diseases, an eosinophilia has been found. It is probable that in these few cases there is a causal connection; even if this were definitely shewn to be the case it would be immaterial as far as the practical use of the blood conditions in the diagnosis of ankylostomiasis, trichinosis or the like is concerned. In normal children Zappert³ found that the eosinophiles were distinctly increased in a number of cases: of 18 cases 6 were over 5 p.c. and one of these reached 14 p.c. Though subsequent observers⁴ have found that in new-born children the eosinophiles rather often reach and exceed 10 p.c., yet in later years any such increase which we cannot attribute to some known cause seems to be at least infrequent⁵. Zappert did not take into consideration the possibility of the action of worms and it is hardly likely that all the children examined were free from intestinal parasites. In the same way it is necessary to

¹ *Journ. Trop. Med.* 1901, p. 285.² J. Ewing, *op. cit.* p. 471.³ *Zeitschr. f. klin. Med.* xxiii. 1893, p. 244.⁴ A. O. M. Fehrsen, *Journ. of Physiology*, xxx. 1903, p. 326.⁵ *Brit. Med. Journ.* 1903, II. p. 1267: J. L. Morse, *Boston Med. and Surg. Journ.* cxlvi. 1903, p. 573: A. Raybaud, *C. R. de la Soc. de Biol.* lvi. (1904), p. 540.

exclude the action of known causes before attributing the increase of eosinophiles seen in the insane to their mental condition alone. The insane as a class are particularly prone to become infected with worms owing to their filthy habits, though it seems fairly clear that a moderate increase of eosinophiles accompanies some instances of several nervous and mental disorders¹. In considering the remarkable eosinophilia which he finds to be universal among the natives in S. China, Horder, though recognizing the possible influence of leprosy and intestinal parasites, does not adduce any evidence that these factors can be excluded.

It is hardly necessary in the present connection to mention myelogenous leukaemia as a disease which is constantly accompanied by an absolute and usually by a relative, eosinophilia, since the blood-film is characteristic in so many other ways.

From this sketch of the occurrence of eosinophilia it may be concluded that it is of materially frequent occurrence with (1) true paroxysmal asthma, (2) dermatitis herpetiformis, (3) pemphigus, (4) *Bilharzia*, (5) *Trichina*, (6) *Ankylostoma*, and possibly with (7) *Filaria*; that it *may* occur with many skin diseases and with any worm (though *Trichocephalus* and *Bothriocephalus* are doubtful); and that one must be prepared to meet with occasional cases which do not fall under any of these heads.

Discussion of the results obtained.

My results shew that 94 p.c. of cases of *Ankylostoma* infection have an eosinophilia of more than 8 p.c. with an average of 18 p.c., and the preceding section indicates that there are comparatively few affections which give rise to figures which are comparable either in magnitude or frequency. As far as miners in active work are concerned, several of these diseases can be very easily excluded since the fact that the man is working is an adequate proof that he is not materially affected with *e.g.* trichinosis. Pemphigus, dermatitis herpetiformis and true asthma may all be described as diseases which are at once rare and likely to be mentioned by the patient. With *Filaria* and *Bilharzia* infection there is perhaps more danger; the latter is becoming com-

¹ *E.g.* chorea: Zappert (*loc. cit.*) records four cases with 8·7 to 19·5 p.c., and T. R. Brown twelve with 5 to 9 p.c. (*Medical News*, LXXXII. 1903, p. 1117). J. A. Capps (*Amer. Journ. Med. Sci.* vol. CXI. 1896, p. 650) records two instances of definite eosinophilia among 19 cases of general paralysis, but the question of worms is not considered.

paratively common in this country though cases of latent infection are quite rare. Eosinophilia is not a pathognomonic sign of *Ankylostoma* infection or of anything else; but the method, if used with discretion and with an adequate knowledge of the necessary detail, can give results which reach a degree of accuracy which is surprising. Certain points must now be considered in the practical application of the method to technical diagnosis on a commercial scale.

The time occupied in making an examination of a mine by the blood-film method is certainly not more than that necessary to obtain and examine a corresponding number of specimens of faeces. It is true that the method requires more experience and skill than the direct microscopic examination of the stools, but sufficient familiarity with the necessary technique and with the microscopic appearance of blood corpuscles is not difficult to acquire. The preparation of the films alone is very simple, and they can be easily sent for examination to those who are more familiar with microscopical work. Under favourable circumstances it is possible to obtain blood films from 10 per cent. of 400 or 500 men in 20 minutes, and the preparation and examination of these specimens can be completed within 24 hours. The bulk of the specimens is small, and a large number can be carried in the pocket; they can, moreover, be kept in good condition for at least several months if immediate examination is for some reason inconvenient. The actual enumeration of the white corpuscles is laborious, each specimen taking about half-an-hour, sometimes much longer. In but few cases, however, is it necessary to do this and so find the exact percentage of eosinophile leucocytes; a rapid general examination will suffice to tell in nearly every instance whether they are present in normal or in definitely increased quantity. Positive results take a little longer (in microscopical examination) than in the direct examination of faeces, but negative results are reached much more rapidly. There is in this method no chance of fraud, since the specimens are obtained directly by the examiner from the subject, and there is little or no difficulty in persuading men to allow their fingers to be pricked.

There are, however, two serious drawbacks to the method. In the first place, as has been already mentioned, it is of very uncertain value in ascertaining whether an infected subject has been freed from the worms by treatment. Secondly, it cannot yield evidence of *Ankylostoma* infection of a character so positive as would justify the immediate administration of anthelmintic treatment. In every case an *absolute* diagnosis can only be made by finding eggs in the stools. But the

method is not designed, nor is it proposed that it should be used, for this detailed examination of individual cases; it is rather a simple, and I think an accurate, way of investigating a large number of men easily and quickly to see if a suspicion of *Ankylostoma* is thereby raised of sufficient strength to justify the troublesome process of obtaining specimens of stools from a number of men. If specimens of blood from 10 or 20 per cent. of the underground men fail to reveal any cases of an increase in the eosinophile cells, it may be assumed that the mine is free from any extensive infection. If, on the other hand, any cases of eosinophilia are met with, the individual source of each blood sample is known; and further investigation of each positive case is then made to see whether the abnormality is due to the *Ankylostoma* or to one of the other causes of eosinophilia. It will be readily seen that the method is most superior to the primary examination of faeces in cases where there is no *Ankylostoma* infection.

The Leucocytes in infected and normal Miners.

The present inquiry has afforded an opportunity to collect information relating to the leucocytes other than the eosinophiles. The average differential counts for each of the groups of men dealt with in the earlier part of this paper are as follows¹.

A. <i>Infected</i>	No. of cases	Lympho- cytes	Inter- mediates	Large hyalines	Neutro- philes	Eosino- philes	Mast cells
Known to have eggs	61	18.7	7.4	3.5	51.9	17.8	0.68
A. Dolcoath, 1902	22	18.8	6.4	3.8	50.5	19.7	0.76
B. „ 1903	24	22.9	14.3	1.7	46.8	13.4	0.91
C. „ 1904	41	25.9	6.1	2.0	44.5	20.7	0.74
Total	148	21.1	7.9	2.9	49.1	18.2	0.73
Calculated after de- duction of 15.7 p.c. eosinophiles	}	25.2	9.4	3.5	58.5	2.5	0.87
B. <i>Normal</i>							
C. Talke	40	29.4	6.8	2.4	58.8	1.9	0.67
C. Snailbeach	42	18.8	17.4	2.2	58.7	2.1	0.79
B. Levant	41	30.3	12.1	1.3	51.8	3.7	0.76
Other cases	35	26.5	10.5	2.4	57.6	2.4	0.57
Total	158	26.1	12.0	2.1	56.6	2.5	0.70

¹ The series which are marked *A* were examined with cover-glasses, *B* with the cigarette-paper method, *C* with slide-smears, and the rest by a mixture of all three methods.

It would appear from these figures that, after making allowance for and deducting the excess of eosinophiles, the differential count of miners infected with *Ankylostoma* is approximately the same as that of similar normal persons. The infected miners have rather more neutrophiles and mast cells, distinctly more large hyaline cells, and fewer lymphocytes of all kinds. So little has been recorded about the occurrence and numbers of mast cells that they merit perhaps rather more extended mention, especially in view of their suggested association with eosinophiles.

It has not been uncommon to meet with statements that mast cells are not found in normal blood¹; that they are only occasionally present²; and that "one-half per cent. is the maximum number in health³." These statements are calculated to convey an erroneous impression. The following figures shew the distribution of the mast cells by percentages in the series of cases which form the basis of this paper:

		Percentages of mast cells					
		0	< 0.5	0.5—	1.0—	1.5—	> 2.0
Infected	148	4.3	34.1	32.9	26.1	4.2	2.5
Normal	158	6.9	35.4	34.2	25.9	1.9	2.5

These figures are based on differential counts of 500 leucocytes in 17 cases out of 306 no mast cell was found in the first 500 leucocytes, but in every case one or more were soon discovered by further search. In a large number of films which I have recently examined with this point specially in view I have in no case experienced any difficulty in finding a mast cell. The conclusion to be drawn would seem to be that they are always present in normal blood.

In about 65 p.c. of cases they exceed Cabot's estimate of the maximum possible in health, and in 8 of the 306 persons 2 p.c. or more were counted. As was previously suggested by Haldane and myself⁴, and has been recently pointed out by Goldhorn⁵, the explanation of this discrepancy no doubt lies in a difference of method; Ehrlich's triacid mixture leaves the granules of these cells unstained, and, though the

¹ E.g. W. S. Lazarus-Barlow, *Manual of General Pathology*, ed. 2, 1904, p. 151.

² Following P. Canon (*Deutsche med. Wochenschr.* XVIII. 1892, p. 206) who failed to find any in 9 of 22 healthy persons.

³ R. C. Cabot, *Clinical Examination of the Blood*, ed. 4, 1903, p. 66. P. Ehrlich and A. Lazarus, *op. cit.* p. 76.

⁴ This *Journal*, III. 1903, p. 125.

⁵ J. Ewing, *Clinical Pathology of the Blood*, ed. 2, 1904, p. 168.

shape and tinctorial properties of the nucleus are characteristic, they are easily missed. Jenner's and similar stains bring out the blue-red metachromatic granules very clearly and render the cell very distinct.

That mast cells are somewhat increased in the presence of an increase of eosinophiles is rendered probable by the figures, though the differences are small. Of the infected cases 32·8 p.c., while of the normal cases only 30·3 p.c. reach 1 p.c. or more: the average for infected men is 0·73 p.c., for normal men 0·70 p.c.; but if allowance is made for the excess of eosinophiles the former figure is increased to 0·87 p.c.

The differential counts obtained for normal miners afford some basis for considering the effect, if any, of their mode of life on the leucocytes. The figures are widely different from those so often quoted from Ehrlich, who places the normal percentage of neutrophiles at 70—72 p.c., while in this series the average is but 57. There is little doubt that the former figure is too high; such a percentage may as a rule be taken to indicate the presence of a definite neutrophile leucocytosis. More recent determinations¹ give lower figures (60 p.c., 66 p.c.); the only accurate determinations made in this country with which I am acquainted are those of A. G. Phear², who obtained the following average figures for normal people—mononuclear 43·4, neutrophiles 54, eosinophiles 1·9, mast cells 0·7. These figures correspond very closely with our own, and lead to the conclusion that the high temperature, coal, tin, arsenic, lead, etc. underground do not cause any marked or definite alteration in the relative proportions of the different varieties of leucocyte.

[*Note.* The classification of the leucocytes adopted here has been previously discussed (vol. III. p. 115). The "intermediates" include non-granular³ mononuclear cells, which are larger and have more cytoplasm and a less densely chromatic nucleus than the typical small lymphocyte, but which have not such large dimensions, such a pale nucleus, or such basophile cytoplasm as the typical large hyaline cell. It is no doubt a very mixed group; and there is some doubt whether the apparent numbers of this variety do not vary with the mode of preparation of the film and the idiosyncrasy of the observer's mood.]

¹ See Bezançon and Labbé, *Traité d'Hématologie*, p. 487.

² *Medico-Chirurgical Trans.* LXXXII. 1899, p. 331.

³ Many of them shew the scattered, irregular granules, staining a metachromatic red with Jenner's stain, which have been recently figured by Ewing, *op. cit.* 1904, Plate III. and p. 123.

Other Worm infections in Miners.

During the numerous examinations of faeces for the presence of *Ankylostoma* which have been made, results of some interest have been obtained with regard to the prevalence of other intestinal worms. Reference has already been made to the occurrence of *Anguillula stercoralis* in some half-dozen cases at Dolcoath (this vol. p. 98). Till recently the Dolcoath specimens were not examined specially for any worms except *Ankylostoma*, and it is to be regretted that much interesting material has in this way been lost. But of recent samples nearly all shew *Trichocephalus* and one or two *Ascaris*. In searching for these other eggs Stiles' plan of repeatedly shaking up the faeces with water and, after a few minutes, pouring off the supernatant fluid has been extensively used; it is very useful, since in some cases the eggs are very few. For *Ankylostoma* eggs it is not so valuable; they are generally very easily found without it, and they do not seem to settle so quickly or so completely as other eggs.

The results obtained are shewn in the following table:

Mine	No. of specimens	<i>Trichocephalus</i>	<i>Ascaris</i>	<i>Oxyuris</i>	<i>Ankylostoma</i>
Levant	25	24	3	1	0
East Pool	23	14	9	1	4
Snailbeach	22	0	0	0	0
Various coal-pits	28	0	0	0	0

No *Taenia* eggs were found in any case. There are we believe no figures published shewing the prevalence of the common worms among an ordinary English adult male population; but the figures of the Snailbeach lead-mine and the Staffordshire coal-pits probably represent approximately what is found among the ordinary population. There is at any rate no reason for supposing that the men in these mines should be less infected than those on the surface; at Snailbeach there is most abundant opportunity for coming in contact with faecal material. Most of the data which have from time to time been published have been recently collected by P. E. Garrison, B. H. Ransom, and E. C. Stevenson¹ from whose table the following figures are extracted:

¹ Bull. No. 13, Hyg. Lab. U.S. Pub. Health and Mar.-Hosp. Serv., Washington, 1903.

*Ankylostomiasis**Percentage of cases infected with*

	<i>Trichocephalus</i>	<i>Ascaris</i>	<i>Oxyuris</i>	<i>Taenia</i>	No. of cases
U. S. Gov. Asylum	10·8	0·4	0·8	0	500
Italy	38	30	4	7	73
India (Dobson)	4·4	10·5	15·4	1·4	1249
„ (Fearnside) ¹	7·3	36·1	—	—	200
Russia	5·0	5·8	7·1	14·5	600
Germany	19·8	16·5	12·4	0·3	2629

These figures can be taken only as rough indications of the frequency of infection. The mode of infection is such that everything depends on the precise habits of life of the persons investigated. Isolated instances of practically universal infection have been recorded: 90 p.c. of Japanese are said to have *Ascaris*, and a similar proportion have been found to harbour *Trichocephalus* in India, while 76 p.c. of Dobson's cases had *Ankylostoma*.

Judged even by a tropical standard, the prevalence of *Trichocephalus* and *Ascaris* at Levant and East Pool is remarkable; taking the two mines together, 79 p.c. of the men harbour the former and 25 p.c. the latter worm. The amount of infection at East Pool is such that the full data may perhaps be of interest:

Sample	<i>Trichocephalus</i>	<i>Ascaris</i>	<i>Oxyuris</i>	<i>Ankylostoma</i>	Earth nematodes and eggs
I	+	+	+		+
II	+	+			
III	+				+
IV	+	+			+
V	+	+			+
VI	+			+	
VII	+				
VIII	nil				
IX	nil				
X	nil				
XI	nil				
XII		+			+
XIII	nil				+
XIV	nil				+
XV	nil				
XVI	+	+		+	+
XVII	+	+		+	+
XVIII	+	+			+
XIX	nil				+
XX	+			+	
XXI	+				+
XXII	+				
XXIII	+	+			
Total	14	9	1	4	12

¹ *Brit. Med. Journ.* 1900, ii. p. 541.

Thus 15 out of 23 samples shewed the presence of some intestinal parasite, while in 6 instances two, and in three instances three were present, the total infections found amounting to 28 in 23 cases.

The figures are also given of the presence of the small nematodes which so frequently enter faeces from the earth; they are found about as frequently in cool non-infected mines such as Snailbeach as they are in the hot Cornish mines. They can be easily found in mud in these mines far from any faecal contamination, but they appear to flourish and multiply better in faeces, in which they often occur in immense numbers. Thus they have been noted at Levant in 7 out of 25 specimens, at East Pool in 12 out of 23, at Snailbeach in 14 out of 22.

Infection with *Ascaris* and *Trichocephalus* is believed on very satisfactory grounds to be direct, and to occur by the ingestion of the eggs themselves. *Taenia* of course requires an intermediate host, and I have not been able to find that cysticercus cysts are specially frequent among Cornish miners¹. It is curious that *Oxyuris* should be so uncommon. It is evident that the thick shells of *Ascaris* and *Trichocephalus* are satisfactorily protective since they flourish in Levant where the water is sufficiently salt to prevent the introduction of *Ankylostoma*. There can be but little doubt that the prevalence of these parasites at Levant and East Pool is directly due to the filthy habits of the men: their absence at Snailbeach may be due to the lower temperature there (62°—68° instead of 70°—90° F.), since it is almost certain that the eggs must contain developed larvae before they will infect if swallowed.

¹ Pork is the chief meat eaten by the Cornish miners.

ON A DYSENTERY TOXIN AND ANTITOXIN.

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I. *Introduction.*

ONE of the most striking points in the pathology of Bacterial Dysentery is the fact that while the bacilli are found regularly in the mesenteric glands, they are not found in the spleen or other organs, and

do not occur in the blood, urine, or milk. In this respect Lentz (p. 320) points out that the disease differs markedly from the septicaemic diseases, such as typhoid, and that it must be regarded as a local infection of the intestinal mucous membrane and corresponding lymphatic glands by the bacillus, the toxin alone passing into the circulation and giving rise to the typical clinical picture, which, as in the case of cholera, gives the impression of a severe poisoning or toxæmia.

The results of experiments on animals with the *Bacillus dysenteriae* (Shiga) bear out this view, as the pathological changes which result from the injection of the living bacillus may be produced equally well by the use of dead cultures; showing that these changes are brought about by some toxic body, and not by any vital action of the bacillus.

The following experiments, which were commenced at the end of 1902, were undertaken with the view of investigating the nature of this toxic body, and in the hope of obtaining an anti-body for it. A preliminary note of the earlier results was published by me in the *British Medical Journal* in December, 1903. At the time the experiments were begun the only reference which could be found in the literature to a soluble dysentery toxin was the statement by Lentz (p. 328) in the article on dysentery in Kolle and Wasserman's *Handbuch der pathogenen Microorganismen*, "that the filtrates of dysentery cultures are toxic." Subsequently, however, Gay (xi. 1902) showed that vaccines prepared by killing the dysentery bacillus by heat and tricresol, or by tricresol alone, undergo on keeping an increase in toxicity accompanied by a shortening of the time necessary to produce death. This is attributed to a process of breaking up of the bacterial bodies.

Conradi (1903) also stated that he was able by a process of "aseptic autolysis" to extract from the dysentery bacillus a soluble toxin, and shortly after this Neisser and Shiga were able to obtain a similar toxin by suspending the bacillus in saline, killing by exposure to heat, keeping the suspension for two days at 37° C., and then filtering. Finally Rosenthal also found that by growing the bacillus in Martin's alkaline broth highly toxic filtrates are formed.

In the present experiments the following bacilli were used:

1. *B. dysenteriae* Shiga.
2. " Kruse (obtained from Král).
3. " Flexner (obtained from Král).
4. " Flexner (Adult Dysentery, Philippines).

5. *B. dysenteriae* Duval (Summer Diarrhoea, Baltimore).
6. " Duval (Summer Diarrhoea, New York).
7. " Eyre No. 1 (Asylum Dysentery, England).
8. " Eyre No. 2 " "
9. " Eyre No. 3 " "

These cultures, with the exception of Nos. 2 and 3, which were obtained from Král of Prague, were very kindly sent me by Professor Flexner and Drs Shiga, Duval, and Eyre.

The toxigenic power of each of these strains was tried, but for most of the experiments Kruse's bacillus was employed, and where the strain of the bacillus is not specified this bacillus is referred to.

II. *Production and Characters of the Toxin.*

The filtrates from ordinary broth cultures of the bacillus are only slightly toxic for animals, but it was found that by using a more alkaline medium much more highly toxic filtrates could be obtained.

For the production of the toxin the ordinary alkaline broth as employed for the production of diphtheria toxin (that is, broth which has been made just alkaline to litmus and then has had added to it 7 c.c. of normal caustic soda per litre) was planted with the bacillus; grown for a varying period at 36° C., and then filtered through a Pasteur-Chamberland filter and the filtrate tested on rabbits by intravenous injection. At first the cultures were grown for ten days before filtration. The following table shows some of the results:

TABLE I. *Toxins obtained by growing the Bacillus for Ten Days in Alkaline Broth.*

No. of toxin	Bacillus	M.L.D. for large rabbits
No. 1	Kruse	1 c.c. *
" 2	"	"
" 3	"	"
" 4	"	"
" 5	"	"
" 6	Shiga	"

* In the tests the minimal lethal dose (M.L.D.) was only approximately determined, that is, the toxin killed at 1 c.c. and not at 0.5 c.c.

Later it was found that by growing the cultures for a longer period much more toxic filtrates resulted, as shown in the following:

TABLE II.

No. of toxin	Bacillus	M. L. D. for large rabbits
K. 8	Kruse	0·10 c.c.
K. 9	„	0·10 „
K. 11	„	0·10 „
K. 15	„	0·10 „

The best results, as regards the toxicity of the filtrates, appears to be reached in about a month to six weeks, and after this the toxicity begins to fall. The filtrate from a three months' old culture was found to be incapable of killing large rabbits at 0·5 c.c. though this dose gave rise to paralysis and diarrhoea. Experiments were made with a bouillon containing twice the amount of alkali but this did not give any increase of toxicity. The toxin in the filtrate is moderately stable—a toxin which had been kept at room temperature for $4\frac{1}{2}$ months being still highly toxic—and is not destroyed by heating at 70° C. for 1 hour, though exposure to 80° C. for an hour seems to entirely destroy it. It is precipitated by ammonium sulphate; thus a filtrate which killed rabbits at 0·1 c.c. intravenously was precipitated with ammonium sulphate, the precipitate dissolved in distilled water and again precipitated. After drying *in vacuo* over sulphuric acid the final precipitate killed large rabbits at 0·002 grammes as shown in the following table:

TABLE III. *Precipitated Toxin (K. 12) tested on Rabbits intravenously.*

Dose in grammes	Weight of rabbit in grammes	Result
0·10	1050	Dead (24 hours)
0·01	880	Dead (3 days)
0·004	1250	Dead (2 days)
0·002	1480	Dead (4 days)
0·002	1700	Recovered after paralysis
0·001	1600	Lived, no paralysis
0·001	1600	Lived, slight paralysis

The results of the intravenous injection of a lethal dose of the toxin in a rabbit are practically the same as when the living, or dead bacillus is used. After a latent period, varying from one to three or four days, according to the amount of toxin injected, severe diarrhoea sets in with paralysis of the limbs, beginning sometimes in the fore, and sometimes in the hind limbs, but ultimately affecting both, as well as the muscles of the trunk and neck. The animal rapidly loses weight, and death occurs in from one to four or five days. *Post mortem* the lesions

resemble those following the injection of the bacillus. There is marked congestion of the large intestine, which may show small haemorrhages and contains a slimy fluid. The lungs show patches of congestion and often small haemorrhages, the other viscera do not, as a rule, exhibit any marked naked-eye changes.

In the rabbit the toxin acts most energetically when given intravenously, but the same result follows subcutaneous, intramuscular or intraperitoneal injections if large doses are employed.

The variation in the susceptibility of different animals to the toxin is very striking. The rabbit and horse are highly sensitive, the latter particularly so, while the guinea-pig, rat, mouse and monkey are hardly affected; thus, 0.1 g. of the precipitated toxin K. 12 (v. Table III), *i.e.*, 50 minimal lethal doses for a large rabbit, when injected subcutaneously in a guinea-pig caused no symptoms beyond transient local swelling and loss of weight.

III. *Immunisation of Animals.*

Attempts to produce an antitoxin by immunisation of rabbits were entirely negative, the serum of an animal which had received a considerable amount of toxin and which agglutinated the bacillus at 1 in 100 showing absolutely no antitoxic power. A goat was immunised with increasing doses of the toxin, beginning with 0.1 c.c. and rising to 200 c.c. The serum of this animal, taken on the 13th day after the last injection, was tested against the toxin on rabbits and showed distinct, though slight, antitoxic power. The animal was bled again on the 33rd day after the last injection and the serum from this bleeding was found to be much more highly antitoxic. This result possibly explained the want of success in the case of the rabbit when the bleedings had been carried out on the 9th day after the last injection and probably before the antitoxic power had reached its maximum. A horse was then immunised with the toxin. The details of this immunisation are interesting as showing the great susceptibility of this animal to the toxin. The toxin used was a very weak one, 1 c.c. being required to kill a rabbit when given intravenously.

The immunisation was begun in December, 1902, by giving 0.5 c.c. of this toxin subcutaneously and injections of increasing quantities were made every 3rd day for a period of 6 weeks when a dose of 150 c.c. was reached. After a dose of 2 c.c. of the toxin the horse had a rise of temperature (102.2° F.) the same evening, and the next day had very pronounced diarrhoea, and was off his feed. Later on, after a dose of

8 c.c., diarrhoea again appeared, but with these exceptions the animal appeared to stand the immunisation well. On the 5th day after the final injection of 150 c.c., however, complete paralysis of the hind legs occurred and the animal had to be killed.

A second horse was then immunised, beginning with a dose of 0.1 c.c. of a toxin of the same strength and very cautiously increasing the doses, which were repeated at intervals of not less than a week. In this case the immunisation was quite satisfactory, no symptoms being shown beyond the transient local swelling and rise of temperature following the injection. Finally, a dose of 400 c.c. of a toxin which killed a large rabbit at 0.1 c.c. was reached, after which the animal was allowed to rest for a month and then bled.

For purposes of comparison a third horse was immunised, and in this case the bacillus alone, *i.e.* without its soluble toxic products, was used. Kruse's bacillus was grown for twenty hours on neutral agar and the growth emulsified with saline. At the beginning of the immunisation this emulsion was heated for half-an-hour at 70° C., and given subcutaneously, beginning with a dose corresponding to one-tenth of 1 tube and gradually rising to 32 tubes. After this an emulsion of the living bacillus was given intravenously until a dose of 10 agar tubes of living culture was reached. One month after the last injection the horse was bled.

The subcutaneous injections did not cause any abscess formation though there was considerable swelling, the intravenous injections caused much more trouble, and after the final dose of 10 tubes of the living culture the animal was very sick, dunging and staling repeatedly with the anus patent and exuding fluid. These symptoms, however, passed off in a couple of days.

IV. *Action of the Immune Serum.*

On comparing the action of the serum of the horse (No. 2) with that of a normal horse by mixing the respective sera with the toxin and injecting the mixture intravenously in rabbits it was at once evident that while normal serum has practically no antitoxic action the immune serum is highly antitoxic. This is shown in the following table (Table IV) which gives the results of one of a large series of tests which have been carried out with this serum. In carrying out the test 1 c.c. of a toxin, the minimal lethal dose of which for a half-grown rabbit was one-fourth of a cubic centimetre (*i.e.*, about 4 M.L.D.), was mixed with varying

quantities of the serum, the mixture made up in each case to 3 c.c. and after standing for half-an-hour in the incubator at 36° C., injected intravenously in rabbits. In the earlier experiments control tests were always made with normal horse serum but as it was found that this had no appreciable protective action these were subsequently omitted.

TABLE IV. *Testing Antitoxic Power of Serum of Horse immunised with Dysentery Toxin.*

1 c.c. Toxin = 4 M.L.D.

No. of rabbit	Weight of rabbit in grammes	Toxin	Serum	Result
1.	2080	0·15 c.c.	—	Lived, after severe paralysis
2.	1930	0·20 „	—	Dead (8 days), after severe paralysis and diarrhoea
3.	2010	0·25 „	—	Dead (2 days)
4.	2915	1·0 „	0·02 c.c.	Lived, no symptoms
5.	2380	1·0 „	0·01 „	„ „
6.	1375	1·0 „	0·005 „	„ „
7.	1835	1·0 „	0·0025 „	„ „
8.	1775	1·0 „	0·0014 „	„ „
9.	1245	1·0 „	0·0010 „	„ „

TABLE V. *Testing Antitoxic Power of Serum of Horse immunised with Toxin (Kruse).*

1 c.c. Toxin = 20 M.L.D.

No. of rabbit	Weight of rabbit in grammes	Toxin	Serum	Result
1.	560	1 c.c.	0·005 c.c.	Lived, no symptoms
2.	620	1 „	0·002 „	Lived, no symptoms
3.	630	1 „	0·001 „	Dead (2 days)
4.	690	1 „	0·0005 „	Dead (2 days)

As the limits of the protective power were not reached in the first series of experiments (Table IV) a further test was made with a much stronger toxin. The result of this is shown in Table V, where it will be seen that the serum has a very powerful antitoxic action, one five-hundredth of a cubic centimetre being sufficient to neutralise 20 minimal lethal doses of the toxin when the two are mixed and kept together for a time before injection.

The prophylactic action of the serum is particularly interesting. This is shown in Tables VI and VII. It will be seen that if the serum be given intravenously it protects absolutely against large doses of the toxin given half-an-hour later. If, however, the serum be given 24 hours before the toxin it does not protect or shows only very slight

protection. This may be due to the excretion or destruction of the antitoxin during the intervening 24 hours. If the toxin be given intravenously in one ear the animals can be saved by an injection of the serum into the other ear five minutes later.

TABLE VI. *Prophylactic Action of Immune Serum. (Serum given intravenously in right ear; toxin (K. 8) given intravenously in left ear half-an-hour later.)*

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin (K. 8)	Result
		Immune		
1.	1345	4.0 c.c.	0.5 c.c.	Lived, no symptoms
2.	1655	4.0 „	1.0 „	„ „
3.	1705	4.0 „	2.0 „	„ „
4.	1710	4.0 „	2.0 „	„ „
5.	1705	4.0 „	2.0 „	„ „
6.	1715	4.0 „	4.0 „	„ „
		Control		
7.	1670	4.0 „ normal	0.5 „	Dead (20 hours)

TABLE VII. *Prophylactic Action of Immune Serum. (Serum given intravenously in right ear; toxin (K. 8) given intravenously in left ear 24 hours later.)*

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin	Result
		Immune		
1.	1635	4.0 c.c.	2.0 c.c.	Lived, transient paralysis in forelegs
2.	1370	4.0 „	2.0 „	Dead (4 days) after paralysis and diarrhoea
3.	1555	4.0 „	2.0 „	Lived, transient paralysis in forelegs
4.	1650	4.0 „	4.0 „	Dead (2 days)
		Controls		
5.	1970	4.0 „ normal	0.5 „	Dead (40 hours)
6.	1695	4.0 „	0.5 „	Dead (20 hours)
7.	1470	4.0 „	0.5 „	Dead (20 hours)
8.	1740	4.0 „	1.0 „	Dead (40 hours)

In the earlier experiments with the antitoxic serum certain irregularities were observed in its action, a given amount of serum sometimes protecting an animal completely and at other times, though saving the animal, failing to completely avert the paralysis and diarrhoea. This was subsequently found to be due to the fact that the toxin and antitoxin require a certain time for their combination. Thus, when 3 c.c. of toxin were mixed with 0.5 c.c. of a somewhat weak serum and

the mixture injected immediately into the ear vein of a rabbit the animal died in four days after severe paralysis and diarrhoea. When the same mixture was kept in the incubator for an hour before injection the animal showed no symptoms. In order to determine the action of temperature on the time of combination two parallel series of experiments were made. In these the toxin and serum were brought to the desired temperature, mixed and kept at this temperature, for varying times, after which they were injected intravenously into rabbits. The results are shown in Tables VIII and IX.

TABLE VIII. *Showing time necessary for combination of Toxin and Antitoxin at 37° C.*

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin	Serum and toxin mixed and injected after	Result
1.	1805	0·5 c.c.	3 c.c.	Immediately	Recovered after severe paralysis
2.	1725	0·5 "	3 "	5 minutes	No symptoms
3.	1525	0·5 "	3 "	15 "	"
4.	1500	0·5 "	3 "	60 "	"

TABLE IX. *Showing time necessary for combination of Toxin and Antitoxin at 0° C.*

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin	Serum and toxin mixed and injected after	Result
1.	1550	0·5 c.c.	3·0 c.c.	4 minutes	Dead (32 hours)
2.	1480	0·5 "	3·0 "	8 "	Dead (42 hours)
3.	1700	0·5 "	3·0 "	15 "	Dead (3 days)
4.	1280	0·5 "	3·0 "	30 "	"
5.	1300	0·5 "	3·0 "	60 "	"
6.	1640	0·5 "	3·0 "	2 hours	Lived, no symptoms
7.	1250	0·5 "	3·0 "	5 "	" "
8.	990	0·5 "	3·0 "	10 "	" "
9.	1655	0·5 "	3·0 "	20 "	" "

From these it is seen that whereas at 37° C. the combination of toxin and antitoxin is complete in less than five minutes; at 0° C. this combination requires a time of between one and two hours.

The fact that the action of dysentery toxin is not abolished by the presence of an equivalent amount of the antitoxin if the mixture is injected immediately on mixing is due to (1) the toxin and antitoxin requiring a certain time for their combination and (2) the free toxin becoming very rapidly fixed by the tissues.

Both these facts are in accordance with our experience in the case of other toxins. The credit of first drawing attention to the conditions

of neutralisation, in respect both of time and of amount of toxin and antitoxin, belongs to Fraser (1896), who investigated these points in the case of venom and antivenin. These conditions were further investigated by Martin and Cherry (1898) for venom and diphtheria toxin, by Ehrlich (1897) and by Knorr (1897) for tetanus toxin.

The rapid fixation of dysentery toxin by the tissues of the body is entirely similar to the fixation of tetanus toxin as pointed out by Dönitz (1897).

The antitoxin is very stable and is not destroyed by exposure to a temperature of 65°—66° C. for 1 hour.

From the foregoing it is evident that old cultures of the dysentery bacillus contain a soluble toxic body which is capable of giving rise in the horse to a powerful antitoxin, and the question naturally arises as to whether this toxic substance is to be regarded as a soluble toxin secreted by the bacillus into the surrounding fluid as in the case of diphtheria and tetanus or is it rather to be looked upon as more properly an intracellular toxin which has soaked out of the cell. There appears to be a large balance of evidence in support of the latter view, thus old cultures (4—6 weeks) which must contain a large number of dead and macerated bacilli are much more toxic than younger cultures (8 to 12 days) when more of the bacilli are alive. This is the reverse of what obtains in the case of diphtheria and tetanus.

Again, by autolysis of quite young cultures of dysentery highly toxic filtrates can be obtained, as was shown by Conradi (1903) and also Neisser and Shiga (1903). In order to ascertain if the toxin obtained by the autolysis of young cultures is the same as that found in solution in old alkaline broth cultures a toxin was prepared from a young agar culture by Neisser and Shiga's method. It was found that 1 c.c. of this (*i.e.*, 20 minimal lethal doses) was completely neutralised by 1 c.c. of the serum of horse No. 2, which had been immunised with the filtrates from old alkaline broth cultures, showing that the toxin is the same whether obtained from the autolysis of quite young cultures or from the growth of the bacillus for some weeks in alkaline broth. Further evidence in the same direction was obtained from the immunisation of horse No. 3, which had received only young agar cultures. The serum of this animal was tested against a toxin obtained in the usual manner by growing the bacillus in alkaline broth. The result is shown in Table X, from which it will be seen that this serum was more powerfully antitoxic than that of horse No. 2, which had received large doses of the toxin (*cf.* Table IV), one-thousandth of a cubic centimetre of the

serum completely neutralising twenty minimal lethal doses of the toxin.

TABLE X. *Testing Antitoxic Power of Serum of Horse immunised with Kruse's bacillus.*

1 c.c. Toxin = 20 M.L.D.				
No. of rabbit	Weight of rabbit in grammes	Toxin	Serum	Result
1.	870	1·0 c.c.	0·1 c.c.	Lived, no symptoms
2.	670	1·0 „	0·03 „	„ „
3.	750	1·0 „	0·01 „	„ „
4.	650	1·0 „	0·003 „	„ „
5.	680	1·0 „	0·001 „	„ „
6.	610	1·0 „	0·0005 „	Died (2 days)
7.	570	1·0 „	0·0002 „	„

If the dysentery bacillus is grown for 24 hours on neutral agar and the growth emulsified in saline and killed by exposure to chloroform vapour for an hour the emulsion of the dead bacilli is highly toxic for rabbits, killing them with the same symptoms as the living bacillus.

A large series of experiments was carried out in order to ascertain if the serum of horse No. 2 (immunised with the toxin only) was capable of protecting rabbits against the dead bacillus. The serum and suspension were given as follows:

- (1) Serum and suspension mixed and given intravenously.
- (2) Serum intravenously followed after half-an-hour by the suspension also intravenously.
- (3) Serum intravenously—suspension subcutaneously at the same time.
- (4) Serum intravenously—suspension intraperitoneally at the same time.
- (5) Serum subcutaneously—suspension intravenously the following day.

In all the above series control experiments were made with normal serum and it was found that while the control animals all died with the typical symptoms in every case, the animals receiving the antitoxic serum were entirely protected.

V. *Comparison of the toxins obtained from bacilli of various strains and the relation of these toxins to the antitoxin.*

The formation by *B. dysenteriae* Kruse of a soluble toxin which can be neutralised by antitoxin suggests an interesting method of attacking

the question of the identity, or otherwise, of the various races of bacilli which have been described in connection with epidemics of bacterial dysentery and allied diseases. With this view nine races of bacilli were examined. The comparative agglutination of these with the serum of horse No. 3 (which had been immunised with Kruse's bacillus only) is shown in Table XI, from which it is seen that the bacilli fall into two classes:—one class, more highly agglutinated by the serum, including bacilli of Shiga, Kruse, and the three strains isolated from cases of Asylum Dysentery in England by Eyre (1904), and a second class which are less highly agglutinated, consisting of Flexner's Philippine dysentery bacillus, and two races of bacilli isolated by Duval from cases of summer diarrhoea in Baltimore and New York. The action of the bacilli on mannite (Lentz, 1902, p. 559) agrees completely with the agglutination tests, as none of the members of the first class cause any alteration in the reaction of the medium, while the four less highly agglutinated races constituting the second group all cause definite formation of acid.

TABLE XI. *Agglutination of Dysentery Bacilli with Serum of Horse No. 3 (2 hours at 36° C. and then over-night at room-temperature).*

	1/100	1/200	1/500	1/1000	1/2000	1/4000
Kruse (Král)	+++	+++	++	+	0	0
Shiga (Shiga)	+++	+++	++	+	0	0
Flexner (Dysentery, Philippines)	+	+	0	0	0	0
Flexner (Král)	++	+	0	0	0	0
Duval (New York, Summer Diarrhoea)	++	+	0	0	0	0
Duval (Baltimore, Summer Diarrhoea)	++	+	0	0	0	0
Eyre (No. 1, Asylum Dysentery)	+++	++	++	+	0	0
Eyre (No. 2, Asylum Dysentery)	+++	++	+	0	0	0
Eyre (No. 3, Asylum Dysentery)	+++	+++	++	+	0	0

An examination of the toxigenic power showed entirely similar results. The filtrates from one month old cultures in alkaline broth in the case of Shiga's bacillus and the three Asylum dysentery bacilli were all toxic as shown below :

TABLE XII. *Toxicity of Filtrates of Alkaline Broth Cultures (1 month old) for Rabbits (intravenous).*

	Dose	Result
B. dysenteriae Shiga	0.2 c.c.	Died (3 days)
Asylum Dysentery Eyre (No. 1)	1.0 „	Died (2 days)
„ „ (No. 2)	1.0 „	„
„ „ (No. 3)	1.0 „	„

all the animals showing the typical paralysis.

The effect of these toxins could, moreover, be entirely neutralised by the antitoxic serum of horse No. 2 (which had received the toxin of Kruse's bacillus only), using from four to ten minimal lethal doses of the various toxins. On the other hand the filtrates from the American bacilli in doses of 5 c.c. intravenously did not affect the animals with the exception of causing diarrhoea, which in some cases was very marked.

TABLE XIII. *Toxicity of Filtrates of Alkaline Broth Cultures (1 month old) for Rabbits (intravenous).*

	Dose	Result
B. Flexner (Philippines)	5 c.c.	Lived, diarrhoea, no paralysis
B. Flexner (Král)	5 „	Lived, no diarrhoea or paralysis
B. Duval (Baltimore)	5 „	Lived, diarrhoea, no paralysis
B. Duval (New York)	5 „	Lived, no diarrhoea or paralysis

These results would appear to support the views of Martini and Lentz (1902), but, on the other hand, they might be explained by the fact that in the case of the American bacilli examined the pathogenic power may have fallen to a very low value, or that, as suggested by Gay (1903), the bacilli belonging to the two types are members of a closely related group of organisms. The latter view is supported by the fact demonstrated by Gay that the antidysenteric serum is effective in different doses for both types of the microorganism.

The formation of a very definite toxin by the bacillus isolated by Eyre from a case of Asylum dysentery in England and the fact that this toxin is neutralised by the antitoxin prepared by the use of the toxin from Kruse's bacillus is particularly interesting.

The comparative action of the sera resulting from the immunisation of horses with the toxin, and with the bacillus on the infection of rabbits with the living bacillus, as well as the comparative bactericidal action of these sera *in vitro*, is at present under investigation.

The fact that the serum of animals immunised with the dysentery bacillus possesses powerful antitoxic properties appears to have been overlooked by previous workers. Shiga in 1901 published the results of his experiments with a serum obtained by immunising animals with the dysentery bacillus, and this serum was used clinically in a large number of cases with excellent results. Kruse (1903) and Gay (1902) working under Flexner also prepared a serum in the same manner and obtained good results. Both Shiga and Kruse appear to have attributed the protective action of the immune serum to its bactericidal action,

and Shiga (1903) points out that this is the first serum used for therapeutic purposes in man which fulfils the conditions laid down by Ehrlich in the Croonian Lecture of 1900, viz., that it contains an immune body for which a suitable complement exists in human serum. Shiga regards his clinical results as supporting Ehrlich's view, but the question of the serum being antitoxic, as well as bactericidal, does not appear to have been considered.

Gay (1902) showed that his serum protected against single lethal doses of vaccine, but though in his second paper (1903) he says that bacteriolysis is not an index of the protective power *in vivo* of antidyenteric serum he makes no statement as to the important antitoxic properties possessed by this serum¹.

CONCLUSIONS.

1. Old cultures of *B. dysenteriae* Kruse in somewhat highly alkaline broth contain a soluble toxin.

2. The same toxin is also contained in the bodies of the young bacilli.

3. The horse and rabbit are very highly susceptible to this toxin, the guinea-pig, rat, and mouse being very resistant.

4. Immunisation of the horse, either with the soluble toxin from old alkaline broth cultures, or with the bodies of the young bacillus, gives rise to an antitoxin.

5. The antitoxic power of the serum of horses so immunised may reach a very high value—in the case of an animal immunised with the bacillus one-thousandth of a cubic centimetre of the serum being sufficient to protect a small rabbit against twenty minimal lethal doses of the toxin.

6. The antitoxin is capable of protecting animals, either when mixed with the toxin, or when given separately at another part of the body either at the same time or shortly before or after the toxin.

7. The toxin and antitoxin require a certain time for their combination *in vitro*, and this time is dependent upon the temperature,

¹ Note during preparation. In a paper published quite recently Rosenthal (1904) describes a serum which he has obtained by injecting horses with both the toxin and cultures; this serum possesses both antitoxic and bactericidal properties, and he has been able to use it in the treatment of 157 cases of bacterial dysentery with most encouraging results.

varying from less than 5 minutes at 37° C., to between one and two hours at 0° C.

8. Shiga's Dysentery bacillus and three strains of a bacillus isolated from cases of Asylum Dysentery in England by Eyre were found to yield a similar toxin, and this toxin was neutralised by the antitoxin prepared by means of the toxin from Kruse's bacillus. A strong point in favour of the identity of the above bacilli.

9. Attempts to obtain a soluble toxin from *B. dysenteriae* Flexner (adult dysentery, Philippines) and from two races of the bacillus isolated from cases of summer diarrhoea by Duval in Baltimore and New York were unsuccessful.

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SOME RESEARCHES ON THE ETIOLOGY OF DYSENTERY IN CEYLON.

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DYSENTERY is, with the exception of Malaria, the disease most prevalent in Ceylon, and the mortality caused by it surpasses by far that due to any other cause. From the reports of Dr Perry, P.C.M.O. of the Colony, one sees that the total number of cases treated in all hospitals during the year 1902 was 3,017, with 999 deaths. In 1901 the number of cases was 4,177, with 1,543 deaths. The deaths due to Malaria were only 115 in 1902, and 89 in 1901; the deaths caused by Enteric Fever were 63 in 1902, and 74 in 1901.

At the suggestion of Dr Perry, to whom I am very much indebted for having facilitated in every way my researches, I have undertaken some investigation into the etiology of the disease in Ceylon. The researches have been carried out on cases treated in the Borella Convict Hospital or in the General Hospital of Colombo. I wish to express my thanks to Dr Johnson, Dr de Silva, and Dr Fernando, who were in charge of the patients. It is well known how different the opinions of authors are regarding the etiology of dysentery. At present the general tendency is to admit two forms of dysentery, an amoebic form, incorrectly called also tropical dysentery, and a bacterial form. Whether this last form is caused always by the same organism or not is not settled, though the most recent researches tend to show that the largest number of cases are due to the *Bacillus dysenteriae* described by Shiga and Kruse. Some authors recently have pointed out quite a different origin. I may mention as an example "La Dysenterie spirillaire" of Le Dantec. According to him in several cases of dysentery the mucus passed in the motions is practically a pure culture of Spirilla. He has never been able to cultivate them. A form of dysentery of children, or at any rate of a disease very nearly related to dysentery (Colitis

contagiosa of Escherich), common in some parts of Italy, is caused probably by the *Bact. coli dysentericum* of Celli.

The so-called "asylum dysentery" is due according to Durham to a very minute micrococcus; while Kruse believes it to be caused by bacilli very nearly related to the bacillus of dysentery.

Methods.

In my researches I adopted the following methods. Stools as soon as they were passed were collected in large sterile Petri dishes. The patients were instructed to pass their stools directly into these dishes, taking care that no admixture with urine took place. The stools were examined as soon as possible; very often as soon as passed. Fresh microscopical preparations for amoebae and other protozoa were constantly made. The warm stage was very seldom used, as in Ceylon there is no need for it. Stained preparations (using dilute fuchsin, and Gram's method) were made at once so as to afford some idea of the bacterial flora present. Agar and gelatine plates were made from flocculi of muco-pus. The best method is to pour the melted agar and gelatine into Petri dishes, let it set, and then smear the surface with a floccule of the pus. With the object of detecting *B. dysenteriae* any typhoid-like colony was examined according to the usual methods for the determination of bacteria. Inoculations in different media were made, using especially glucose-agar to see whether the germs isolated gave rise to gas production or not. I have examined 23 cases of dysentery by the above methods and with the following results.

Case I. K. 66, Borella Convict Hospital; acute case; death.

At autopsy the mucosa of the colon and the rectum was thickened, covered with muco-pus, and studded with a large number of very small superficial roundish ulcers.

The stools were twice examined during life; they contained practically no faecal matter and had no faecal odour. There was a large amount of pus and some blood. The microscopical examination showed a large number of leucocytes and red blood corpuscles. No amoebae or other protozoa could be seen. Some non-motile bacilli and some cocci arranged in pairs and chains were present. Several cover-slip preparations were stained with Löffler's methylene blue and by Gram's method. With methylene blue the cocci and bacilli were very well stained, and a few appeared to be inside the leucocytes. All the bacteria appeared not to stain by Gram, with exception of a few cocci and some rare bacilli. These last were large and rather thick, I failed to grow them. Several gelatine and agar plates were prepared from the stools. After 24 hours many colonies were present. In agar some of these were smaller, more delicate, somewhat translucent. In gelatine also some colonies were more delicate than the others and typhoid-like. Hanging

drop preparations from these colonies showed a non-motile, rather short, plump bacillus. From twelve of the more delicate colonies and twelve of the coarser and more opaque ones stab cultures were made in as many tubes of sugar agar. Eight tubes out of 12 of the first row did not show any evidence of gas production. The other four as well as the whole of the second row presented abundant gas bubbles. From the tubes without gas, inoculations were made in broth, gelatine agar, litmus milk, litmus agar, etc.

The organism isolated showed all the cultural characters of the Kruse's bacillus, the description of which will be given later. It was well agglutinated (1:40) by the blood of the patient and also by the blood of several convalescents from dysentery. The blood of the patient likewise showed agglutinating power when tested upon a culture of *Bacillus dysenteriae* (Kruse), which I brought from Kruse's laboratory.

The bacillus isolated from the stools of the patient was inoculated subcutaneously (1 c.c. of broth culture) into two rabbits. One died after 3 days, the other after 5 days—neither of them presented any symptom of dysentery. At autopsy the spleen was a little enlarged, and the mucosa of the whole intestine was somewhat congested, but no ulcers were present. No lesions could be detected in the other organs.

Case II. K. 9110, Borella Convict Hospital; acute case; recovery.

The stools were examined 25. I. 1904, the patient having been ill three days. Much pus and very little blood. Kruse's bacillus was isolated by the usual method. The patient's blood did not agglutinate the bacillus until the 29. I., i.e., on the seventh day of the disease. On the same date the faeces, which had become nearly normal in the meantime, were again examined: Kruse's bacillus could not be found. Agar and gelatine plates showed only abundant colonies of *B. coli* and some rare colonies of a coccus. 16. II. 1904, the stools were examined a third time: appearance completely normal. *B. dysenteriae* not found. *Amoeba coli* could not be found in this case though looked for on several occasions.

Case III. Sinhalese boy in General Hospital under care of Dr Fernando; subacute case; recovery.

Stools examined 12. II. 1904. Very little faecal matter, no faecal odour, mucus in large quantity, little pus and blood. Microscopically many leucocytes; rare red blood corpuscles. No *Amoebae coli* or any other protozoa. *B. dysenteriae* found. The bacillus easily agglutinated (dilution 1:40, time 2 hours) by the blood of the patient after the first 10 days of the disease and also by the blood of several people recovering from dysentery.

Case IV. Adult Sinhalese, No. 1419, Borella Convict Hospital; acute case; recovery.

Stools examined 29. I. 1904. Large amount of pus, very little blood, no faecal matter. Microscopically many leucocytes, few red blood corpuscles, no faecal detritus, *Amoeba coli* absent. Kruse's bacillus found.

Case V. No. 3383. Banda, Borella Convict Hospital; acute case; recovery.

Stools examined 31. I. 1904. No faecal matter, no faecal odour, large amount of muco-pus, and very little blood. Microscopical examination: large number of leucocytes, very few red blood corpuscles, no ova of worms, no amoebae or other protozoa. Some non-motile bacilli were seen. Stained coverslip preparations show

some plump, rather short bacilli, also cocci in pairs and short chains, both organisms staining by Gram. Agar and gelatine plates incubated for 24 hours at 37° C. showed colonies of *Bacillus coli* and of a *Streptococcus* which slowly liquefies gelatine (*Streptococcus coli gracilis* Escherich ?) besides a few colonies of *B. dysenteriae*.

Case VI. Mary, General Hospital ; chronic case ; died on 27. I. 1904.

At autopsy the mucosa of colon and rectum found thickened, showed a number of large irregular ulcerations with thickened edges. Several hepatic abscesses. Scrapings from the ulcers showed many *Amoebae*. Kruse's bacillus not found. Cultures from the intestinal contents yielded *B. coli* and *B. pyocyaneus*. The pus of abscesses did not show amoebae. Cultures therefrom showed no growth.

Case VII. K. 32, Borella Convict Hospital ; subacute case ; recovery.

Stools examined 2. II. 1904 contained no faecal matter, and possessed no faecal odour. Large amount of mucus, some pus and very little blood. Microscopical examination showed numerous leucocytes, some of which enclosed red blood corpuscles. Some free corpuscles, no crystals, no ova. *Amoebae coli* as well as other protozoa absent. Coverslip preparations treated with dilute Ziehl's stain (1 : 4) showed a large number of bacilli, some of which were rather long and others rather short and plump. Some very fine spirilla are also present ; cocci in pairs and short chains can be seen. Some of the cocci are contained in leucocytes. Preparations by Gram's method show the cocci and a few bacilli well stained. *B. dysenteriae* found, but many of the colonies found in the agar and gelatine plates were those of *B. coli*, of a streptococcus liquefying gelatine slowly (*Str. coli gracilis*) and of a large spore-forming motile bacillus also liquefying gelatine and probably belonging to the *B. subtilis* group. The spirilla could not be grown. *B. dysenteriae* isolated from the stools was readily agglutinated by the patient's serum after the fifth day of the disease.

Case VIII. I. 4589, Borella Convict Hospital ; chronic case.

Stools contained no blood ; only a little pus and mucus. Large amount of faecal matter. Microscopical examination showed much faecal detritus, many ova of *Trichocephalus dispar*, some leucocytes and rare blood R. C. No *Amoebae coli*. *B. dysenteriae* not found. The plates showed only colonies of gas-producing organisms. The patient's serum readily agglutinated the Kruse bacillus.

Case IX. Karuppen. General Hospital.

The bacteriological examination was made from the intestine after autopsy 2. II. 1904. Mucosa of caecum, colon and rectum thickened, covered with muco-pus and presenting many small superficial roundish ulcers. Spleen slightly enlarged. Nothing to be noted as regards other organs. *Amoebae coli* absent. *B. dysenteriae* found. From the spleen and blood of the heart no germs could be grown.

Case X. No. 9601, unconvicted, Borella Convict Hospital ; subacute case.

Stools examined 8. II. 1904, the patient having been ill for 6 weeks. Stools consist practically of muco-pus and blood only. *Amoebae coli* absent. *B. dysenteriae* found.

Case XI. Menatshe. General Hospital ; chronic case.

The bacteriological examination was made from intestine after autopsy 9. II. 1904. Mucosa of colon and rectum thickened and studded with many small roundish ulcers. *Amoebae coli* absent. *B. dysenteriae* found.

Case XII. Patient admitted into Borella Convict Hospital 20. II. 1904. Pulse 112. Temperature 102° F. Complained of griping pains. Frequent motions with blood and pus. Recovery after a few weeks.

Stools examined 20. II. 1904. No faecal detritus; great number of red blood corpuscles and leucocytes; no protozoa; no ova; a few non-motile bacilli and some rare cocci. A bacillus very similar to Kruse's was isolated from the stool, this bacillus showed some slight differences which will be described presently.

Case XIII. Prisoner 125 (Daniel), Borella Convict Hospital; acute case; recovery.

Stools composed of a very large quantity of mucus, some pus, and very little blood; faecal odour entirely absent. Microscopical examination; many leucocytes, some red blood corpuscles, *Amoebae coli* absent. Coverslip preparations stained with dilute fuchsine (1:4) show a large number of short, plump bacilli, some of which are grouped in clumps. *B. dysenteriae* found. It is remarkable that in this case the colonies found in the plates contained a large majority of Kruse's bacillus, while colonies of *B. coli* were extremely rare.

Case XIV. Borella Convict Hospital; acute case; recovery.

Stools examined 6. IV. 1904, were passed by the patient directly into a Petri dish and examined at once. They consisted of muco-pus and a very little blood. *Amoebae coli* absent. Coverslip preparations stained with dilute carbol-fuchsine show some plump short bacilli and some spirilla. By Gram's method bacilli and spirilla are decolorized. Kruse's bacillus found. Plates showed some colonies of *B. coli* and other gas-producing organisms. The spirilla could not be grown.

Case XV. T. 6185, Borella Convict Hospital; acute case.

Stools passed by the patient directly into a sterile Petri dish and examined at once. Very little faecal matter; faecal odour absent; large amount of mucus; very little pus or blood; a few ova of *Trichocephalus dispar*; *Trichomonas intestinalis* present; *Amoebae coli* absent. Kruse's bacillus present. The blood of the patient taken on the 3rd day of illness did not agglutinate Kruse's bacillus, neither the original one nor any strain isolated by me from other cases; agglutination positive after 7 days of illness.

Case XVI. Kelavanti. General Hospital.

Bacteriological examination made from intestine after autopsy. Mucosa of small intestine greatly injected, that of colon swollen and studded with many small superficial ulcers. A large amount of muco-pus covered the mucosa. The examination was made from flakes of the muco-pus. No protozoa were found. *B. dysenteriae* present.

Case XVII. Dr H..... Colombo General Hospital, Paying Ward; chronic case. Suffered from his first attack of dysentery some years ago. After that an abscess of the liver developed, which was successfully operated on. A few months ago another attack set in; again an abscess of the liver developed. The patient died two days after abscess operation.

Stools examined 17. IV. 1904. There was some faecal matter with muco-pus and blood. Microscopically a large number of *Amoebae* were found. These amoebae were of large dimensions (50—80 μ) and slowly emitted blunt pseudopodia. Many *Trichomonas* were also present and also some other protozoa to be described

afterwards. Kruse's bacillus not found. The agar and gelatine plates showed only gas-producing organisms. Serum reaction with Kruse's bacillus was always negative with any dilution of the blood (1:2, 1:20, 1:40) using the original strain as well as any other Ceylon strain isolated by me.

Case XVIII. T. 6124, Borella Convict Hospital; acute case; recovery.

Stools examined 23. iv. 1904, consisted practically of pus, mucus and blood. Faecal odour completely absent. The microscopical examination showed numerous leucocytes, red blood corpuscles and a few non-motile bacilli. *Amoebae coli* and other protozoa absent. *B. dysenteriae* was grown easily, the greater majority of the colonies in the agar and gelatine plates being colonies of this bacillus while the colonies of *B. coli* were exceptionally rare. Agglutination positive with any strain of Kruse's bacillus.

Case XIX. K. 6285, Borella Convict Hospital; acute case; recovery.

Stools examined 8. iv. 1904. A very large amount of mucus, little pus and blood; no faecal matter; faecal odour completely absent. *Amoebae coli* absent. *B. dysenteriae* found.

Case XX. Prisoner No. 7592, Borella Convict Hospital; acute case.

Stools passed 4. v. 1904, showed large amount of mucus, some blood and pus. Microscopically many leucocytes, some red blood corpuscles and a few non-motile bacilli were observed; but no amoebae or other protozoa. *B. dysenteriae* was found alone in gelatine and agar plate cultures made from the stools.

Case XXI. H. 8949, Borella Convict Hospital; acute case.

Stools consist of mucus and a little pus and blood. No faecal odour whatever. Microscopically some leucocytes and a few red blood corpuscles were seen. Stained preparations show a few cocci and some rare bacilli. *Amoebae coli* absent. *B. dysenteriae* present.

Case XXII. K. 3856, Borella Convict Hospital; acute case.

Stools examined 10. v. 1904. Large amount of blood and pus; no amoebae. *B. dysenteriae* present.

Case XXIII. K. 6262, Borella Convict Hospital; acute case.

Stools examined 10. v. 1904. No faecal matter; no faecal odour; much mucus and a little blood. Microscopically many leucocytes and a very few red blood corpuscles found. Some rare short non-motile bacilli were seen. Amoebae and other protozoa absent. *B. dysenteriae* present.

To sum up, I have examined 23 cases of dysentery occurring in Ceylon. In 19 of these cases the presence of *B. dysenteriae* Shiga-Kruse was demonstrated in the intestinal contents or stools. In one case the bacillus could not be grown, but the blood of the patient agglutinated the bacillus. In one instance (Case XII.) a bacillus closely resembling Kruse's bacillus was grown.

In two instances (Cases VI., XVII.) a large number of *Amoebae* were found in the stools, whilst the presence of *B. dysenteriae* Shiga-Kruse could not be demonstrated.

*Morphology and biology of the Shiga-Kruse Bacillus
as found in Ceylon Dysentery.*

The discoverers of the *B. dysenteriae*, Shiga in Japan, and Kruse in Germany, differ slightly in their descriptions of the organism. The most important difference refers to its motility. Kruse described the germ as non-motile. Shiga on the other hand described it as motile. Flexner also admitted some degree of motility, at least in young cultures. Vedder and Duval state that they never came across any motile strain, but nevertheless have been able to detect flagella. I can say at once that as regards my cases in Ceylon all the strains I have grown were absolutely non-motile, and although using different staining methods, I could never detect flagella. As regards the morphology of the Shiga-Kruse bacillus, it is a rather short, plump bacillus, easily stained by the usual aniline dyes. It is not stained by Gram's method. In cultures all the strains isolated by me in Ceylon behaved in the same way as the original strain from Germany. In broth the bacillus gives rise to a general uniform turbidity without any formation of a pellicle on the surface, or of gas bubbles. The indol reaction is always negative. It does not produce gas in glucose-agar, nor does it coagulate milk. In gelatine plates, in Piorkowski media, in Elsner potato-gelatine, the colonies always show a great resemblance to those of *B. typhosus*. In agar plates the colonies are often more delicate and smaller than those of *B. coli*, and they are somewhat translucent. Still, some strains of *B. coli* form colonies in agar absolutely identical with those of the Shiga-Kruse bacillus. According to my experience litmus-milk cultures after 15 hours show very little acidity—not enough to distinctly alter the colour of the medium, and after 3 to 5 days the milk becomes alkaline. I have never seen any coagulation. It is evident from this description that a great resemblance exists between the dysentery and the typhoid bacilli. Practically they cannot be distinguished by their cultural characters—though they can be easily distinguished by the fact that Kruse's bacillus is certainly non-motile. Further, the serum of an animal immunized against typhoid has no action on Kruse's bacillus, and *vice versâ*. Another point of resemblance, in addition to many others, between the Kruse and the typhoid bacillus is, according to my experience, in the production of haemolysins (see *Lancet*, 1902, February 15th). Both organisms are capable of forming a haemolysin which produces complete solution of dog's erythrocytes. The maximum amount of haemolysins is found in filtrates of 2 weeks' old cultures. The

bacillus does not produce spores. It possesses but little resistance to heat and to the usual fluid disinfectants.

Pathogenicity. My experiments were carried on with guinea-pigs and rabbits only, using strains of *B. dysenteriae* isolated in Ceylon as well as cultures brought by me directly from Prof. Kruse's laboratory, in Bonn. I have not observed any appreciable differences as regards the pathogenicity of the various strains. Intraperitoneal inoculations of 1 c.c. of broth culture kill guinea-pigs in 15 to 20 hours, a much smaller dose is sufficient to kill the animal by sub-dural inoculation. Rabbits inoculated hypodermically waste very rapidly and seldom live longer than 6 to 8 days. Sometimes experimental animals may suffer from violent diarrhoea, but in my experience the stools never contain blood or pus. At autopsy the intestinal mucosa is generally congested; I have never seen any ulcerations. These results do not correspond with the experiments of Vaillard and Dopter (*Presse Médicale*, No. 39, 1903), who claim to have reproduced dysentery in rabbits by means of sub-cutaneous injections of the Shiga-Kruse bacillus. On the other hand my experiments perfectly tally with those of Kruse himself, and with the experiments I carried on in his laboratory when he discovered the organism.

Agglutination. The technique was the same as that usually employed. Generally dilutions of 1:40 were employed and the preparations examined after two hours. All the strains from Ceylon and Germany behave practically in the same way, with the exception of the strain isolated from case XII (see p. 505). The blood shows presence of specific agglutinins only after the first 5–8 days of the disease. The agglutination is generally well marked in convalescents. I have not data enough to justify a statement as to how many months the blood of the patient retains the agglutinating power. In a case 4 months after the disease had been cured agglutination was still very marked. The agglutination in several cases is not regular, the reaction seems to be sometimes almost intermittent. One day with the dilution 1:40 there is a very good agglutination; the day after with the same dilution there is no agglutination at all, though there may be perhaps with a dilution of 1:10 or 1:20. The reaction is rarely positive using a dilution of more than 1:150.

The intensity of the agglutination does not proceed parallel to the severity of the disease; the agglutinating power of the blood may drop very low, *sub finem vitae*. The blood of healthy persons never agglutinated any of the strains even in dilutions of 1:10. The blood of patients

suffering from diseases other than dysentery generally did not show any agglutinating power as regards Kruse's bacillus, with the exception of one case of typhoid fever and one case of malaria. In the former the reaction was positive up to a dilution of 1:30, in the latter up to a dilution of 1:20. Both patients denied having ever suffered from dysentery. In dysentery then, as in typhoid fever, it is advisable to use a dilution of not less than 1:40.

Immunization and Vaccination. So far I have conducted few experiments in this connection. I have tried to immunise rabbits by subcutaneous inoculations of live cultures, or of cultures killed by exposure for one hour at 75° C. Using live cultures the rabbits constantly died after a few days. Treated with dead cultures two rabbits lived for about one month; but their sera showed only slight agglutinative power. Two weeks after inoculation the serum when diluted to 1:200 agglutinated the Shiga-Kruse bacillus, but the protective power of the serum was practically *nil*. Even 2 c.c. of the serum added to the minimal lethal dose of Kruse's bacillus was not able to save a guinea-pig of 250 grms. No dissolution of the bacilli could be noticed in the peritoneal liquid extracted in the usual way by means of fine capillary tubes. The results are quite like those I obtained in Germany, probably better ones would follow if I could use donkeys and horses. Kruse has succeeded in immunizing horses and in getting a powerful serum, which has given apparently fairly good results in the treatment of several cases. Shiga also and others have succeeded in preparing sera of high protective power by different methods. As regards the preparation of an anti-dysenteric vaccine this I think might be worth a trial in localities where severe epidemics of dysentery are frequent. Kruse inoculated himself and two of his assistants subcutaneously with 1 c.c. of a broth culture, previously kept for an hour at a temperature of 65° C. The inoculation caused some slight elevation of temperature and a certain degree of malaise. These symptoms disappeared soon. There was also some local reaction at the seat of inoculation. After a few days the serum agglutinated the dysentery bacillus. It was therefore to be surmised that a certain degree of immunity had been acquired.

Prevention of dysentery. Though one cannot deny that drinking may convey the germ, it must be noted that here in Colombo the water is from a bacteriological point of view a very good one. This also holds for the water used in the gaols—where cases of dysentery are so common. I think the best way to diminish the number of cases of dysentery is to improve the sanitation—especially in regard to the removal of excreta,

drainage, etc. In Germany it has been observed that outbreaks of dysentery occur often in places which have a good supply of water but a bad system of drains. Patients with dysentery should be isolated and their stools disinfected at once. Another possible means of prevention (I am speaking of course of the bacterial form of dysentery) would be an anti-dysentery vaccination. This is worthy of trial, but does not appear to me promising.

Observations on the Intestinal Bacterial Flora of Dysentery Cases.

B. dysenteriae is usually found associated with several germs in cases of dysentery. In fact it is exceptional—personally I have met only with one such case—to find the dysentery bacillus alone, *B. coli* is, in my experience, almost constantly present; generally, even when very fresh stools are examined, the majority of colonies grown on gelatine and agar plates are those of *B. coli*. If the stools are not examined very soon after they have been passed the *B. coli* multiplies to such an extent that it becomes very difficult to isolate the dysentery bacillus. I have grown various strains of *B. coli*—all alike in their more important cultural characters (coagulation of milk, etc.). Most of these strains were non-motile; some sluggishly motile; a very few actively motile. The blood of rabbits immunized with one strain did not show agglutinative power for the other strains. Some of the strains were not agglutinated at all by the blood of the patients, a few were agglutinated up to a dilution of 1:20 and one 1:30. But they were agglutinated to the same extent or very little less by the blood of perfectly healthy persons. The blood of typhoid patients agglutinates them in some cases more than the blood of dysentery cases. The possibility of normal sera, typhoid sera, etc., agglutinating certain strains of the colon bacillus has been experimentally demonstrated by Iatta and other authors including myself. Iatta has shown that the serum of an animal immunized with the typhoid bacillus besides agglutinating this bacillus agglutinates also—though to a very much less extent—some strains of coli; moreover he has demonstrated that a certain parallelism may be observed between the two agglutinations, viz. if the agglutinative power of the blood for the typhoid bacillus increases, it increases also for those strains of the colon bacillus. No importance then can be attached to the fact that the sera of some dysentery patients may agglutinate some strains of *B. coli*.

In two cases a bacillus producing a green pigment with all the characters of *B. fluorescens liquefaciens* was grown. In two cases

organisms of the *subtilis* group were present. In three cases anaerobic motile bacilli with characters similar to *B. enteritidis sporogenes* Klein were isolated. In several instances coverslip preparations made directly from the pus in the stools and stained with dilute carbol-fuchsin or methylene blue showed Spirilla of two types: some were finer, longer and rather faintly staining, others were thicker, shorter and appeared deeply stained; both types were decolorised by Gram's method. I never succeeded in growing them. Cocci not decolorised by Gram's method were present very often in coverslip preparations made from the muco-pus; though in the agar and gelatine plates I could seldom obtain colonies of cocci. As a rule these cocci—in broth cultures—appeared arranged in chains. Some strains of these streptococci liquefied gelatine. Among the liquefying strains some in broth cultures possessed chains composed of very small individuals ($0.2-0.4\mu$): *Streptococcus coli gracilis* of Escherich. In two cases diplococci were grown having practically all the characters of *Streptococcus lanceolatus*. Blastomycetes were very rarely observed.

None of these different germs had anything to do with the etiology of the disease; their inconstant presence in dysentery, and their frequent occurrence in other diseases, as well as occasional presence in normal individuals prove this, though it is possible that in some cases they may modify the course of the disease by acting as secondary infectious agents.

Paradysentery.

Paradysentery.—From case XII, which clinically did not differ from an ordinary acute case of the disease, I grew a bacillus which culturally can scarcely be distinguished from the Shiga-Kruse bacillus. The only cultural peculiarities—to which very little importance can be given—are its more abundant growth on agar, its production of indol, and its more marked production of acids. The typical Shiga-Kruse bacillus grown in litmus-milk forms very little acid, so that the colour of the medium is only very faintly changed and the medium becomes alkaline shortly after. With the strain grown from case XII the colour of the litmus-milk was changed to red in 12 hours and has remained so for over two months. There was no difference otherwise between this germ and Kruse's bacillus: the milk was never coagulated, and no formation of gas took place in sugar agar. The behaviour of this strain as regards agglutination was very interesting. It was very well agglutinated by the blood of the patient, but it was never agglutinated by the blood of

any of the other cases of dysentery I tested with it. The blood of case XII never agglutinated any other strain of dysentery bacillus to any appreciable degree, as will be seen by reference to the following table.

TABLE I. *Blood from Case XII. Agglutination with different strains of dysentery bacillus.*

Date from onset of illness	Strain isolated from stools of patient XII.	Strain from Germany	Strain from Case I.	Strain from Case III.	Strain from Case VII.
3rd day	1 : 10	—	—	1 : 10	—
7th day	1 : 30	—	—	—	—
10th day	1 : 100	—	—	1 : 10	—
18th day	1 : 100	—	—	1 : 10	—

Time of observation 2 hours.

1 : 10, 1 : 20, etc., means that the agglutination reaction is positive up to a dilution of the blood of $\frac{1}{10}$, $\frac{1}{20}$, etc. The sign “—” means that with a dilution $\frac{1}{10}$ the reaction was negative. A less dilution than $\frac{1}{10}$ was not tried.

Bacilli similar or possibly identical to that of case XII have been isolated by Kruse in cases of a peculiar form of dysentery which is endemic in many asylums in Germany. Kruse has called such organisms *Bacilli pseudodysenterici*: the existence of these bacilli has been denied recently by Vedder and Duval, though they have been found again by other authors. These bacilli, according to my experience in Germany, are non-pathogenic to rabbits, but in my case here in Ceylon the strain was pathogenic to rabbits in the same way as the true dysentery bacillus. The subcutaneous inoculation of 0.5 c.c. bouillon culture of the strain from case XII was sufficient to kill rabbits in 4 to 7 days. At autopsy the intestinal mucosa showed marked congestion but no ulcers. I think that as there are cases of paratyphoid—clinically indistinguishable from typhoid (though some authors have pointed out a few slight differences), but due to organisms very nearly related to the *Bacillus typhosus*—so there are cases of dysentery, practically indistinguishable from the ordinary ones, but caused by bacilli very nearly related to the *Bacillus dysenteriae*. Such cases might be termed in analogy Paradysentery and the germ *B. paradysentericus*, of which most probably there are different strains.

Amoebic Dysentery in Ceylon.

From cases VI and XVII neither *B. dysenteriae* nor any organism resembling it could be grown. The intestinal flora was represented by *B. coli* and some cocci. No spirilla were present. In case VI, which was

studied by me only at autopsy, the heart blood was found not to agglutinate any strain of dysentery or paradysentery bacilli. In case XVII the blood was tested several times during life with several strains of dysentery and with the strain of paradysentery always with negative results. The faeces of both cases contained a very large number of amoebae. Both cases presented abscesses of the liver. The pus of the liver abscess of case VI was sterile. Amoebae could not be detected. The pus of the liver abscess from case XVII could not be examined. It is well known how many discussions have taken place on the question of the existence of an amoebic form of dysentery. If authorities like Kartulis, Kruse, Councilman, Manson, Koch, etc., admit it, other authorities like Grassi and Celli deny it. The authors who do not admit the existence of an amoebic form of dysentery base their opinion especially on the fact that amoebae may be found in the stools of perfectly normal persons or in people suffering from other diseases. This is certainly correct, though here in Ceylon out of more than 150 examinations of faeces I have been able to find amoebae only in cases VI and XVII, which I consider cases of amoebic dysentery. In all the cases of bacterial dysentery so far I have never found amoebae.

Does the so-called *Amoeba coli* represent one species only or several species? Celli distinguishes several species of amoebae inhabiting the human intestine; Kruse and Pasquale admit two different species—a harmless one living in the intestine of healthy persons, and a pathogenic one which is the cause of a form of dysentery. Schaudinn recently, after some very interesting experiments, came to the conclusion that under the denomination of *Amoeba coli* two species of amoeba, different morphologically and biologically, are included. The two amoebae, according to this author, present such important differential characters that they might almost constitute two different genera. He has called the one species, which is harmless, *Entamoeba coli* Lösch; the other, which is pathogenic, *Entamoeba histolitica*. Schaudinn states that a distinction between ectoplasm and endoplasm is very difficult in *E. coli*—impossible in the resting amoebae. The nucleus is clearly defined, very distinct and contains several nucleoli. Reproduction takes place by fission and by formation of cysts, each containing eight nuclei. On the other hand in *E. histolitica* the nucleus is very indistinct, and often absolutely invisible. Reproduction takes place always by fission—never by formation of cysts presenting eight nuclei, so characteristic of the *E. coli*.

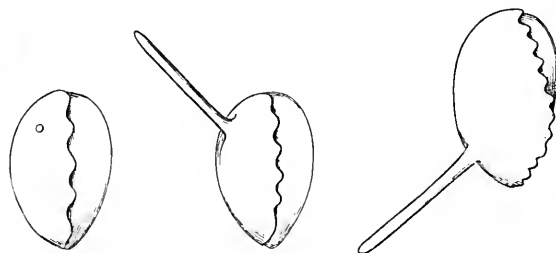
In my two cases (VI and XVII) I noticed that the amoebae

presented a very indistinct nucleus—often quite invisible—and I never observed cysts with eight nuclei. These two characters would point to its being the *Entamoeba histolitica* of Schaudinn.

*Presence of other Protozoa in the Intestine of cases of
Amoebic Dysentery.*

In my cases VI and XVII—besides many amoebae—a large number of *Trichomonas intestinalis* were present. The most of them were of large dimensions, with a pear-shaped or roundish body and very actively motile.

In case XVII, in addition to many *Trichomonas*, I observed several examples of another form of protozoon (see Figure). This was of some-



Appearance of protozoon (*Entamoeba undulans* Cast.) at different intervals—(semi-diagrammatic) stools of Case XVII.

what larger dimensions than *T. intestinalis*, the maximum diameter reaching from 18 to 30 μ . The usual shape was oval. There was an absolute absence of flagella. The organism presented a continuous rapid undulating movement from one to the other extremity of its body, and always in the same direction, this pointing to the presence of an undulating membrane. Now and then at an interval of 15–20 seconds a very narrow long pseudopodium was shot out from the body. Only one pseudopodium was emitted at a time. The pseudopodium was emitted very quickly, and very quickly retracted. The pseudopodium was sometimes protruded from one part of the body and sometimes from another part. The organism had a finely granulated protoplasm; a differentiation between ectoplasm and endoplasm apparently did not exist, the protoplasm being practically of the same character throughout. In a few individuals something like a very indistinct nucleus could be observed, but in most of the specimens no nucleus at all could be seen.

One small vacuole was often present—seldom more than one. The vacuole never possessed the characters of a contractile vacuole. The position of the vacuole varied. The protoplasm never contained red blood corpuscles, but often some bacteria and granules.

Preparations stained by different methods were far from being satisfactory. The sudden death of the patient prevented a more thorough investigation of this protozoon. Apparently it was not a *Cercomonas* or a *Trichomonas*, as flagella were invariably absent. Nor was it one of the amoebae usually met with in the human intestine, seeing that the organism possessed an undulating membrane, and the shape and the mode of emission of the pseudopodia were different from what one sees in *Amoeba coli*, in which the pseudopodia are of large dimensions, blunt, and emitted and retracted rather slowly. In case XVII both organisms and also a large number of *Trichomonas* were present, rendering comparison between all of these parasites easy.

CONCLUSIONS.

From my investigations it would appear that in Ceylon there are several forms of dysentery:

(1st.) By far the most frequent one is the bacterial form due to the *Bacillus dysenteriae*, Shiga and Kruse.

(2nd.) A rare form is due to bacilli very nearly related to the typical Shiga-Kruse bacillus. Such a form might be called paradyentery in analogy to paratyphoid—leaving the term pseudodysentery to denote forms of disease absolutely different from dysentery, as for instance, pseudodysentery from *Bilharzia* (see Low and Castellani, *Zeitschr. f. Schiffs- u. Tropen-Hygiene*, 1904, Band VIII).

(3rd.) A third form of dysentery is represented by amoebic dysentery; and the species of *Amoeba* which causes it in Ceylon is probably *Amoeba histolitica* Schaudinn. This form of dysentery is apparently rare in the island.

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MEDICAL ASPECTS OF THE SWEDISH ANTARCTIC
EXPEDITION, OCTOBER 1901—JANUARY 1904.

By ERIK EKELÖF,

Medical Member of the Expedition.

THE object of the Swedish Antarctic Expedition was to conduct researches relating to the geography and natural history of the Antarctic regions south of Cape Horn. The expedition was to start from Sweden during the autumn of 1901. On the approach of the Antarctic winter, in February or March 1902, some of the party were to be landed in a suitable place in the Antarctic regions, with the object of wintering there. The members remaining on board the ship "Antarctic," were to carry on investigations in the regions around Cape Horn, the Falkland Islands and South Georgia. The next summer, *i.e.* in December 1902 or January 1903, the ship was to return in order to pick up the wintering party at their station; and, thus reunited, the whole expedition was to go back to Sweden, where, as we had calculated, we should arrive in the spring of 1903.

According to this plan the equipment of the winter party was of the greatest importance, as it was expected that this party would be quite isolated for one, perhaps several years, without a chance during this time of renewing their stores. On the other hand the portion of the expedition remaining on shipboard was during the greater part of this time to cruise in regions where new supplies of provisions and other necessary articles could easily be procured.

The issue of the expedition was, however, quite contrary to what had been calculated, and in order to give a clear picture of the conditions under which the different parties of the expedition were afterwards reduced to live, it is necessary to give a short account of the expedition.

Everything went on for the most part as we had calculated up to the southern summer of 1902—1903, when the "Antarctic," as agreed upon, was to fetch off the winter-party. But owing to unfavourable

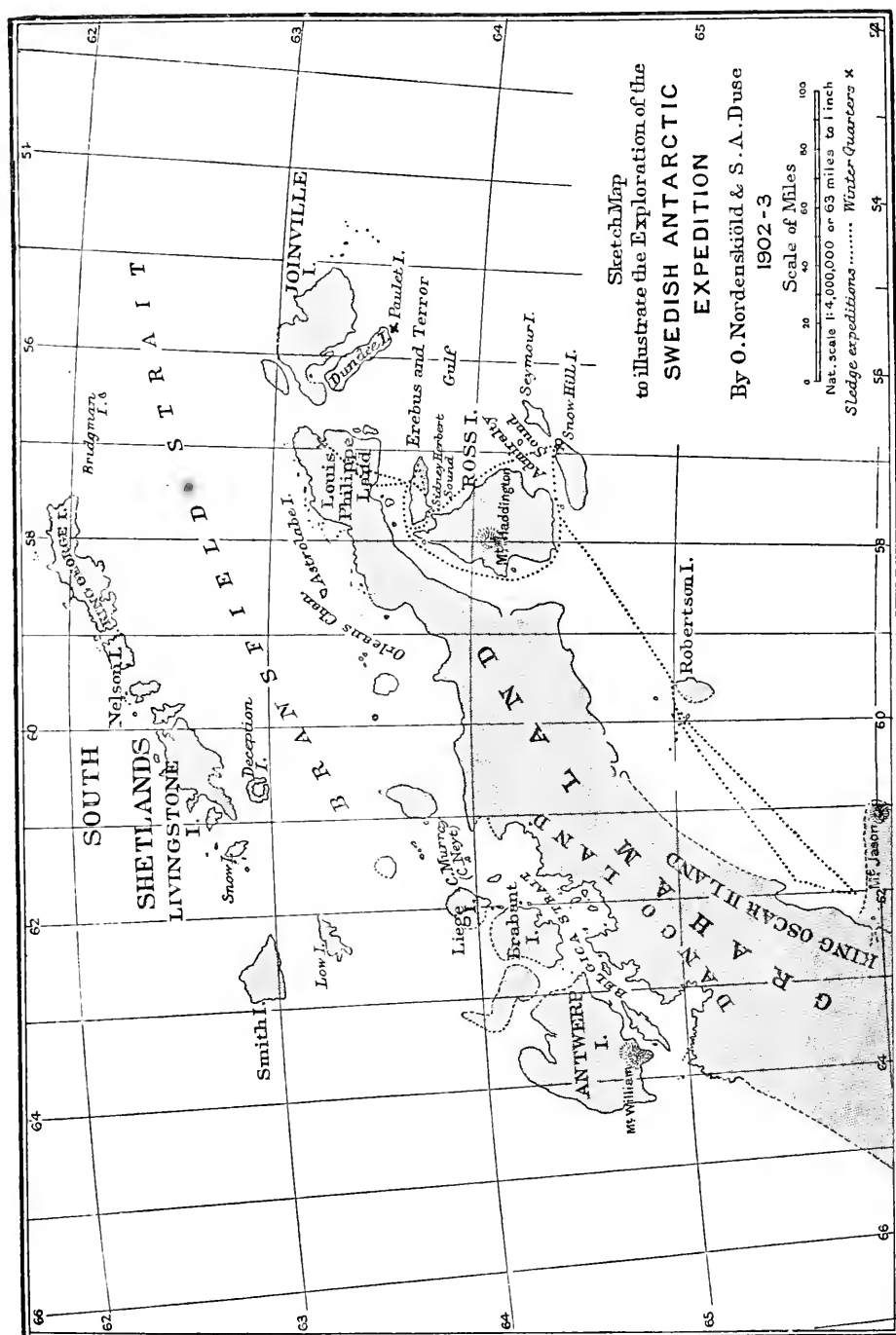


Fig. 1.

conditions of the ice, the "Antarctic" could not, as it had done the previous summer, take the route to the winter station on Snow Hill through the sound between Joinville Island and Louis-Philippe Land. Consequently it was determined to try to reach the winter station simultaneously by two separate ways. Firstly, starting from the vicinity of Mount Bransfield, on the north-east coast of Louis-Philippe Land, a party of three persons, travelling on foot, was to try to reach the winter station, which lay at a distance of about 120 (English) miles. A tent, provisions and other necessities were to be transported on a sledge; the plan being that if the conditions of the ice in the neighbourhood of the winter station appeared too unfavourable to allow of a vessel forcing a passage, the party at the station should join the three men from the "Antarctic," returning with these to the place at Mount Bransfield, where, according to agreement, the "Antarctic" could be expected to fetch off all of them in the course of one or two months. Secondly and at the same time the "Antarctic" was to try to force her way along the north coast of Joinville Island in order to enter Erebus and Terror Gulf to the east of this island, thus making an attempt to reach the winter station in this way. But both of these enterprises failed. The sledge-party met with the most insuperable physical obstacles, and, after marching a week, it was compelled to return to the starting-point. The "Antarctic" on the other hand really succeeded in entering Erebus and Terror Gulf, but was here caught by the drifting ice, sprang a leak during a severe pressure of the ice, and finally sank on the 12th of February 1902. The people on board escaped with difficulty to Paulet Island in the north-east of Erebus and Terror Gulf.

As the winter was rapidly approaching and there was no possibility at this season of reaching the winter station on Snow Hill they could only prepare for wintering on the spot. The sledge-party waited in vain for the return of the "Antarctic," and when the time of waiting, previously agreed upon, was at an end, this party, too, went into winter quarters. In this way the expedition became divided into three different parties, each wintering by itself during the southern winter of 1903. At the winter station, the only one of the three places having resources for establishing communication with the other parties during the winter, nothing was known about the attempts to bring relief and the unfortunate result of these endeavours.

In October 1903 two persons from the party on Snow Hill, during a sledge-journey to the north, accidentally met the three persons who

had wintered in the neighbourhood of Mount Bransfield and who were now making a second attempt to reach the winter station, an attempt, the result of which would have been rather uncertain, if they had not received this lucky help. Frost-bite which had attacked two of the party made the march so slow, that, without succour, it would have been impossible to reach the winter station before the breaking up of the ice shortly afterwards in Sidney Herbert Sound. On the 16th of October 1903 two of the wintering parties were in this way united at the station on Snow Hill.

On the 8th of November the Argentine Relief Expedition under the command of Captain Irizar, on board the gunboat "Uruguay," approached the vicinity of this station, and it was determined that the embarkation should take place on the evening of the following day. In the night between the 8th and 9th of November, however, six men of the party on Paulet Island came to the winter station. On the 10th of November everyone on Snow Hill went on board the "Uruguay," and the following day the 13 persons still on Paulet Island were fetched on board. Thus, on the 11th of November 1903, the whole expedition was reunited, and the journey home was immediately commenced. The expedition arrived at Buenos-Ayres on the 2nd of December 1903, and Stockholm was reached on the 10th of January 1904.

As seen from the foregoing account, the expedition was divided into several separate parties which during well-defined periods were living under somewhat different conditions. In the following pages I shall treat separately the matters of hygienic and medical interest relating to each of these periods.

1. *From leaving Gothenburg, 16 Oct. 1901—to the debarkation of the party which was to winter on Snow Hill, 15 Feb. 1902.*

The crew, consisting of Swedes, Norwegians and two Englishmen, numbered, together with the officers, 20 men; while there were 9 scientific members. The average age of those on board was $27\frac{1}{2}$ years; the 2 oldest were 45, the 2 youngest 18 years old.

About the vessel of the expedition, the "Antarctic," there is little to say; it was rather old (built 1871), but still strong and of the common whaler type. The persons in the gunroom had pretty comfortable quarters; each one had his own cabin. The forecastle on the contrary was small and narrow, in the tropical regions unendurably hot, and in the polar difficult to warm. In the tropics most of the members

of the expedition slept on deck. As usual on such vessels there was much damp which caused considerable discomfort, especially in the Antarctic regions; ice was formed on the inner sides of the hull of the ship, the bedclothes froze fast to the wood and became wet and mouldy.

On the journey out we touched at: Sandefjord (17—19 Oct.), Falmouth (27—28 Oct.), St Vincent, Cape Verde Islands (14 Nov.), Buenos-Ayres (16—21 Dec.), Port Stanley, Falkland Islands (1 Jan. 1902) and lastly (16 Jan.), at Observatory Island also (situated close to the north side of Staaten Island, the projection to the south-east of Tierra del Fuego). We touched at the last-mentioned place in order to compare our magnetical instruments with those in the Argentine observatory on this island.

Very few cases of sickness occurred during the voyage out. We may mention, among others, a case of scabies, probably acquired in Falmouth. In the tropical regions, especially after having begun to use water taken on board at St Vincent, most of the members suffered from diarrhoea, which was happily of a harmless kind and only in few cases needed any treatment. We may also note a case of synovitis (of bursa olecrani). From now on a great number of trivial accidents happened to the personnel: contusions, burns etc., few of which called for treatment, none of those injured being rendered unfit for duty. The health of the human members of the expedition remained on the whole very satisfactory, but this unfortunately cannot be said of the 14 Greenland dogs, taken on board in Gothenburg and intended for use at the future winter station. Through one of these dogs there came distemper into the whole pack, all the dogs with one exception became infected, and 10 died. The rolling of the vessel, the lack of sufficient room for isolating the different individuals, and, lastly and principally, the warmth of the tropics, combined to cause this loss, which was a most severe one for us. Owing to these causes only 4 dogs were left us: 2 males and 2 females. In order to augment the stock of dogs, 8 Falkland dogs were taken on board in Port Stanley, an experiment, however, that totally failed. True enough, these dogs were strong, hardy animals (but still not so good for their special purpose as the Greenland dogs), but they were soon all killed by their four savage relatives from the other side of the globe. These on the other hand later on rapidly increased in number, and on the journey home 9 fullgrown and 6 young dogs of pure Esquimaux-dog race were presented to our reliever, the Argentine Government. The provisions,

brought with us for the dogs, consisted of bread, dried fish, dog-pemmican and "fedte-græves," a kind of very greasy, cheap pemmican, made of offal from slaughter-houses etc.

The wintering party with its stores was put on shore on the island of Snow Hill on the 15th of February 1902. Six days later the "Antarctic" left this place, never more to be seen by those left on shore.

2. *The "Antarctic" Party, 15 Feb. 1902—12 Feb. 1903.*

During the greater part of this time the "Antarctic" remained in the stormy and chilly regions in the vicinity of South Georgia, the Falkland Islands and the southern part of Tierra del Fuego. Part of the medical and surgical stores had been brought on shore at Snow Hill; another part was left on board of the "Antarctic," where the captain, Mr C. A. Larsen, undertook the medical duties during my absence from the vessel. This going on shore of the doctor to live among a party consisting of 6 persons only, may at first glance perhaps seem improper. The cause was however, partly, that the "Antarctic," according to the plan made, was in contradistinction to the land-party to stay in regions where in case of any grave accident or illness medical assistance could be had in 2, or at the utmost in 3 weeks (such places are Ushuaia in the southern part of Tierra del Fuego, Observatory Island, where an Argentine man-of-war is usually stationed, and lastly at the Falkland Islands). Another reason for my leaving the ship was that I should make bacteriological studies which, in accordance with our scheme, had to be made on the Antarctic *terra firma*.

During the sojourn of the "Antarctic" in the aforesaid regions the provisions were sometimes renewed at Port Stanley and Ushuaia. Sometimes the flesh of seals and birds was used for food as well as eggs of penguins and other sea-birds, this especially during the stay in the neighbourhood of South Georgia, during the southern winter 1902. Fish, too, taken during the journey, entered during this period rather liberally into the diet. Plenty of excellent fresh water was to be had everywhere in these regions; in the real Antarctic regions fresh water was procured by melting down glacier-ice or old sea-ice that had lost its salinity.

Captain Larsen only reported one grave case of sickness as occurring during this period, that of our lamented friend and comrade Dr A. Ohlin, the first zoologist of the expedition. He had not been in perfectly good

health at the beginning of the voyage. In the neighbourhood of South Georgia he caught cold, and his illness from this time commenced to grow worse rapidly. He was sent home from Port Stanley on the 22nd of August 1902, and died of consumption in Sweden in the month of July 1903.

3. *The party on Paulet Island, 12 Feb. 1903—11 Nov. 1903.*

This party consisted of Captain Larsen, Mr K. A. Andersson (zoologist), Mr C. F. Skottsberg (botanist) and the whole crew, with the exception of 3 men,—20 persons in all. While the "Antarctic" drifted in the ice in Erebus and Terror Gulf after having been damaged, there was plenty of time to make preparations for all eventualities. All on board tried to render their clothing more suitable for wintering in these regions: sleeping-bags of canvas were sewn; and the provisions were fetched on deck and divided in small packs, convenient for carriage during a journey over the ice. At the date of the sinking of the "Antarctic," on the 12th of February 1903, most of the provisions and a great many other articles, serviceable for the future, had been taken on to the ice, together with two big boats and a smaller one. After struggling against current and drifting ice for 16 days all at last succeeded in reaching Paulet Island (lat. $63\frac{2}{3}^{\circ}$ S. and long. 56° W.), but during this struggle against storm and ice the greater part of the things brought from the "Antarctic" were unfortunately lost.

Of the provisions thus safely brought to Paulet Island, there were during the following winter given to each man daily the following rations: 22·3 gm. dried vegetables, 46·2 centilit. tinned fresh vegetables, 1·5 gm. tinned fruit, 18·4 gm. margarine, 108·7 gm. ship-biscuits, 22·9 beans, peas, barley etc., 2·9 gm. coffee, 1·9 gm. tea, 4·8 gm. cocoa, 4·8 gm. sugar; in addition to this also a little syrup, citric acid, lime-juice, and mustard was consumed. Part of the people had small quantities of tobacco. As may be seen from these figures, the provisions brought on shore from the vessel were in themselves far from sufficient. All had tried, according to agreement, to bring on shore in the first place dried and tinned vegetables, biscuits, coffee, tea and cocoa; it being hoped that animal food could be got on the spot, if necessary. Fortunately Paulet Island was found to be the breeding place of a great colony of Adelie-penguins, shags (cormorants), and also seals. Soon stores of penguin-meat, sufficient for the winter, had been collected, which stores were buried in the snow to preserve them and to prevent them

from being blown away. But of seals only relatively few were found. This was very unfortunate, the only fuel that was to be had in these regions being the blubber of these animals. The result was that it became necessary during the 3 or 4 coldest winter months to restrict the preparation of boiled food to but one meal a day. During this period a very light breakfast was taken about noon-time, this meal consisting only of tea or cocoa and one ships-biscuit. The chief meal of the day was not served before 6 p.m.; it consisted principally of soup, made with seal or penguin-meat, seal-blubber and dried vegetables. During the first part of the winter, as well as after the beginning of August, the seals became more numerous and the diet accordingly more generous; two such daily meals of meat and seal-blubber, with tea, cocoa or coffee, being then taken. During the winter fishing was carried on with good result as often as the weather permitted, the catch making an excellent change in the rather monotonous diet. During the last part of the stay on Paulet Island the penguins had begun laying their eggs, which are said to have been consumed in almost incredible quantities. Excellent fresh water was obtained during the whole winter from a little lake in an old extinct crater; a hole was always kept open here, sometimes through a layer of ice about 1.5 metre thick. For want of salt, sea-water was used in preparing the food. Immediately after arriving at

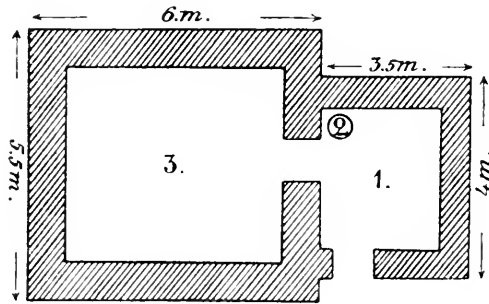


Fig. 2. House of the party at Paulet Island.

1. Store-room and kitchen.
2. Forge used as fire-place.
3. Sleeping-room.

Paulet Island, a house was built. The walls were made double with an intermediate layer of sand and guano. The roof consisted of a sail from the "Antarctic"; later on it was also covered with seal-skins, in the same degree as these were procured by hunting. The house consisted

of two rooms: a smaller, outer one, used as kitchen and store-room, and a larger, inner one, along the walls of which were arranged the sleeping places. In order to keep these free from the water which gathered on the floor from drippings from the ceiling etc., they were raised a little above the level of the floor by a layer of stones, upon which the sleeping-bags were spread. The greater number of these sleeping-bags were made of canvas, a few of guanaco-skin. The sleeping-bags were also made warmer and more comfortable by some woollen blankets which had been saved from the "Antarctic." Furs were not used, but only common woollen clothes.

Want of shoes occasioned great inconvenience, and every possible makeshift had to be resorted to. For instance, galoshes, lined and legged with skin of penguins, were used by some of the men. The cold being severe out of doors as well as in the stone-hut, and most persons being short of clothes, most of the time was passed in the sleeping-bags. Train-oil lamps were used for illumination, making it possible to read some books which had been brought with the party. Playing at cards was a most popular method of killing time.

The cooking was done with the help of seal-blubber, a small forge from the "Antarctic" being used as a fire-place. This forge was a most useful thing, saving time as well as fuel. The greatest inconveniences of the stone hut were damp and darkness. The great amount of hoar-frost formed on the inside of the roof melted when the sun shone outside in calm weather or when cooking was going on indoors, drenching through the men's sleeping-bags as well as their clothes.

In spite of all these most unfavourable conditions a remarkably good state of health was maintained during the whole time. Nevertheless amongst this party, as well as with the two others, there occurred frequent digestive disturbances, such as diarrhoea and colic; everybody being more or less attacked by these symptoms. Rheumatic muscular pains were rather common as well as frost-bitten toes, fingers and parts of the face; and a few cases of snow-blindness also occurred. Only a few of the men having snow-glasses, some protection for the eyes had to be improvised; small frames of wood or wire were made and covered with the thin blue cloth from a Swedish flag. These improvised "snow-glasses" proved to be of very great use.

A peculiar circumstance, which ought also to be mentioned, was the abnormally frequent occurrence amongst all the party of the need of urination. It lasted the whole time and was especially inconvenient during the nights. There may have been some degree of polyuria, but

at all events not in any higher degree. No other symptoms of illness showed themselves in connection with this. As soon as the members of the expedition returned to a more civilized diet, these symptoms at once disappeared. I presume that they stood in connection, partly at least, with certain dietetical conditions of which more will be said later on.

The only grave case of illness among this party was that of the Norwegian sailor Wenersgaard, who, at the time of our departure from Sweden, was a strong healthy man about 20 years of age. This person was attacked by acute articular rheumatism during the stay in the vicinity of South Georgia, August 1902. In March 1903, on Paulet Island, he had a fresh attack. In addition to the other symptoms there also appeared insufficiency of the heart, and after severe sufferings, lasting about six weeks, Wenersgaard at last died on the 7th of June 1903. He was buried in a cairn on an open place on the beach of the island, a simple wooden cross bearing his name and the day of his death being erected over the grave on the arrival of the Argentine Relief-Expedition at the island.

4. *The party at Mount Bransfield, 29 Dec. 1902—16 Oct. 1903.*

This party consisted of only three men: Mr S. Duse, Lieutenant of the Royal Swedish Artillery, cartographer, Mr J. G. Andersson, Lecturer at Upsala University, geologist, and one sailor.

After having vainly tried to reach the winter station on Snow Hill, this party had to return to the place of embarkation. This was situated at Bransfield Strait, near Mount Bransfield, on the north-eastern shore of Louis-Philippe Land (c. $63^{\circ}3'$ S. lat. and 57° W. long.) and is now named Hope Bay. During the first weeks of their stay there the three men lived pretty comfortably upon the provisions brought on land, having no uneasiness about the "Antarctic." But the weeks passed on, and, no news having been received by the end of February 1903, it became evident that some misfortune had occurred, and the party prepared to pass the winter at the place. When leaving the "Antarctic" none of the party had thought it possible for them to be separated both from the vessel and the party at the winter station. The equipment was consequently rather scanty. As a great part of the provisions had been lost in a heavy snow-storm during their efforts to reach Snow Hill, the three men were now at the beginning of the winter in a rather critical situation.

The clothes were not suitable for wintering, but they were improved by being lined with penguin-skins and small pieces of woollen stuff etc. Their sleeping-bags were made of guanaco-skins. A small stone hut was built. The walls were rather thick, 1·3 metres near the ground and at the roof 0·75 metres, while the height of the hut was about that of a man. For roof they had only an old tarpaulin, which unfortunately

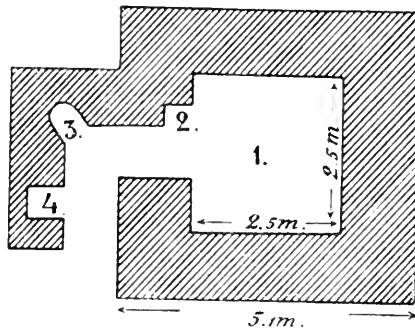


Fig. 3. House of the party at Mount Bransfield.

- | | |
|-------------------|-------------------------|
| 1. Sleeping-room. | 2. Fire-place. |
| 3. W.C. | 4. Depository for meat. |

proved not to be water-tight. They had brought a tent from the "Antarctic," and this tent was raised inside the stone hut; the ground under the sleeping-bags was covered with penguin-skins. A small porch was erected, and in this porch the stove was built. A chimney, or venthole for the escape of the smoke, was made of empty tin-boxes, the bottoms of which had been taken out, the boxes being then pushed into each other. A wood-shutter was used as the outer door, and a seal-skin was suspended as a curtain for the inner part of the hut.

This party too was obliged to use seal-blubber for fuel and lighting, but, happier than the party at Paulet Island, these three men had an abundance of seal during the greater part of the winter. Not far off there was also a colony of penguins, and these two fortunate circumstances saved them from starvation. Here, as at Paulet Island, the party had not a sufficient quantity of salt, sea-water being used for cooking instead. At first the use of this sea-water caused very severe attacks of diarrhoea and colic; these attacks, however, ceased by degrees. On returning to more civilized diet, no constipation or other disturbances of the digestive organs occurred.

The provisions brought on shore from the "Antarctic" were as follows: 225 kg. biscuits, 30 kg. margarine, 95 kg. tinned meat, 105 kg. tinned fish and herring, 10 kg. sugar, 5 kg. coffee, 5 kg. cocoa, 1 kg. tea, 35 kg. tinned soups, 3 kg. dried fruit, 8 kg. condensed milk, 25 kg. barley, 12 kg. dried vegetables, 7 kg. salt. A great part of these provisions having been consumed during the first two months, when the men still expected the return of the "Antarctic," and a rather large quantity being spared for the sledge journey to Snow Hill, no exact information can be given as to the daily rations. Every day during the winter, however, each man was given three biscuits, *i.e.* about 200 gm., and some dried vegetables. Penguin- and seal-meat, together with seal-blubber, was the chief article of diet. A small quantity of fish was also caught.

In spite of all the unfavourable circumstances: damp, cold, darkness, monotonous diet, absence of cleanliness and want of physical and psychical occupation, the state of health of this party too was very satisfactory. No case of severe illness occurred, but only rheumatic muscular pains, slight disturbances of the digestion, snow-blindness and cases of frost-bite. To prevent snow-blindness this party used spectacles like those employed by the Esquimaux, the light being admitted to the eye by a narrow horizontal opening only. This kind of spectacles protected the eyes very well. The appetite and sleep of this party, too, were very good, but the members of this party also complained of the need of frequent urination. At the last successful attempt to reach the winter station, two of the men got their feet frost-bitten, both of them were in a rather bad condition on their arrival at Snow Hill; the frost-bitten feet being much swollen and with large suppurating wounds. After some weeks' treatment they were both quite restored.

5. *The party at the winter station on Snow Hill,*
15 Feb. 1902—10 Nov. 1903.

This party consisted of six persons: Mr O. Nordenskjöld, the leader of the expedition, Lecturer at Upsala University, geologist, Mr G. Bodman, our meteorologist, who had charge also of the magnetical observations, Mr E. Ekelöf, medical officer and bacteriologist, Sr. J. Sobral, Lieutenant of the Argentine Navy, and two sailors.

The winter station was situated in an open space, free from snow, on the island of Snow Hill (c. 64° 22' S. lat. and 57° W. long.).

Materials for the house were brought from Sweden, and its erection was completed after two weeks. The dimensions of the house may be seen from the following plan. The walls were composed of a double

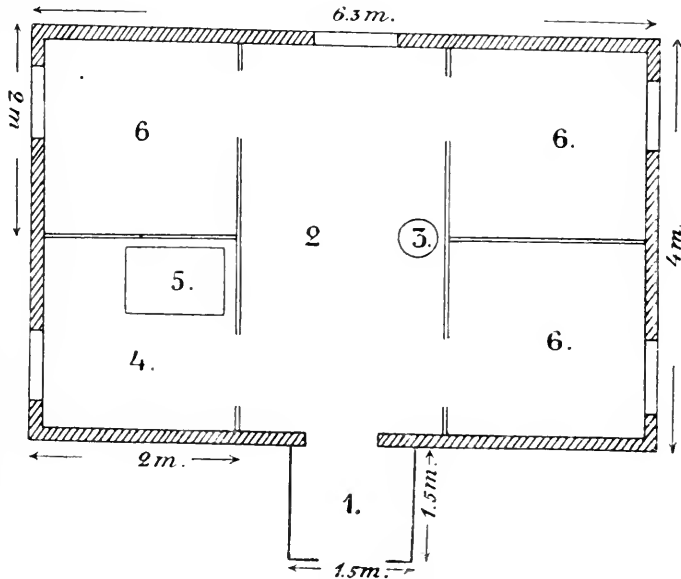


Fig. 4. The house on Snow Hill.

- | | |
|-------------------|------------------------------|
| 1. Entrance. | 2. Working- and dining-room. |
| 3. Stove. | 4. Kitchen. |
| 5. Kitchen-range. | 6. Cabins. |

The height of the rooms = 2.2 m.

layer of rebated planks, with an intervening space about 1.5 cm. in width. The outer as well as the inner side was covered with thick black paste-board, the roof with asphalted paste-board. The floor consisted of the following layers, reckoned from the bottom upwards as follows: first, floor-timbers with a layer of rebated planks, paste-board, and rebated planks again, and upon these latter a thick felt carpet with, finally, a linoleum carpet. A small porch or entrance was built on one of the long sides of the house. The interior of the house was divided into five rooms, there being two small cabins at each gable of the house and a larger room in the middle. This larger room was used as the dining- and working-room; three of the cabins served as bedrooms and the fourth as kitchen. A kitchen-range was placed in the kitchen and

a small stove in the larger room. This stove, as well as the fire-place in the kitchen, was heated with coal during the first year; but later on, when the coal supply was exhausted, with seal-blubber. In the upper part of the house was a small garret, serving as store room. The entrance to this garret was through a hole in the ceiling of the larger room. There was a window to each room. The house was stayed at each of the four corners by thick ropes, and by two thick beams and a large number of provision-boxes, piled up at the lee side of the building (the storms generally blowing in the same direction). The house was built on a small hill, sloping in all directions, so that the gathering of snow and water was avoided.

In the house during the nights everyone used sleeping-bags lined with cotton-wool and covered with guanaco-skin. On sledge-expeditions we used sleeping-bags of reindeer-skin for two or three persons, or sometimes of canvas lined with double blankets for only one person. The party had a good store of all sorts of clothes and furs, but the woollen clothes proved to be best, the furs being rather heavy and clumsy. The furs were used only exceptionally, if someone was obliged to make prolonged observations out of doors, when the temperature was very low; but generally good woollen clothes with special "wind-clothes," as they were called, above them, were sufficient and most comfortable. Many kinds of foot-gear were used, but on long excursions "komager" or shoes made of reindeer skin proved to be best. When living at the station we used reindeer-skin shoes, soled with wood or birch-bark. These latter shoes especially were very warm and flexible; within them we had socks of goat-hair or dried grass. For head-gear, skin-caps (so-called Helsingör-caps) were mostly used. In hard wind or severe cold these caps were pulled down over the ears, and a small piece of woollen stuff could be tied over the face, sheltering nose and cheeks and leaving only small openings for the eyes and mouth.

During the day-time the temperature in the house was generally about $+13^{\circ}$ to 15° C., but during the nights the temperature went down to freezing-point or even lower. The great difference in temperature between the higher and the lower parts of the rooms was rather unpleasant. Close to the floor the temperature was very seldom above the freezing-point, while only 2 metres higher the thermometer stood at $+15^{\circ}$ to 20° C. Thick layers of ice soon were formed on the floor near the walls. In the springtime this ice melted, drenching the whole house, the water standing sometimes several centimetres deep on the floor. To avoid this inconvenience some holes were made in

different parts of the floor to let the water out. In the winter the walls in the mornings were usually covered with hoar-frost, which, melting during the day, soaked through the paste-board, in which there began to grow great quantities of mould. In a very short time the whole house was infected with this mould, destroying clothes, mattresses, books and provisions, and also causing some difficulty in the carrying out of scientific work, as, for example, in the bacteriological researches. At last we were obliged to take away the paste-board; but the mould having gained a firm footing nearly everywhere, it soon became necessary to remove all provisions out of the house into the open air. Paste-board has consequently proved to be unsuitable for lining the inner walls of such a house as ours. The best thing would perhaps be to have the walls merely varnished and without inner covering.

The station being situated at lat. $64^{\circ} 22'$ S. we never had any real polar night. But the cloudy sky and the heavy storms with their drifting snow darkened the atmosphere very much, and as, at the dark part of the year, we could pass only very little time out of doors, we had not much use of the few hours of feeble daylight.

The meteorological conditions were most trying and dangerous. It is true, that no extreme degree of cold was observed, the lowest temperature being about -41° C., and the mean temperature of the year only -11.8° C.; but day after day, sometimes for weeks at a time, there were continuous storms blowing during the winter with a velocity of 15 to 30 metres per second. The greatest velocity observed was 34.7 metres per second. The mean velocity of the wind during the whole year was 8.2 metres per second. At a temperature below -25° C. with a simultaneous wind-velocity of 20 to 25 metres per second, it was practically impossible to remain in the open air for more than a few minutes without getting parts of the face frostbitten. During such severe storms and cold there was nothing else to do but to remain in the little narrow dark house: when overtaken by them in sledging-excursions there was no other resource than camping and waiting, sheltered in the sleeping-bags, until the storm ceased. Uncommonly great and rapid changes of temperature and of barometrical pressure were observed. The greatest difference of temperature in one day amounted to 33.9° C. The barometrical maximum was 761.3 mm., the minimum 708.6 mm. The mean barometrical pressure of the year was 739.9 mm. During about $\frac{2}{3}$ of all the days of the year there was either rain or snow, and we had consequently very little sunshine. During the whole month of June 1902, by the help of a Campbell-Stokes

"sunshine-recorder," we could register only 4·5 hours of sunshine. The humidity of the air was generally rather great.

Very pure fresh water was procured from ice taken from a glacier in the vicinity of the house. During the short period of the year when there was running water this was taken on account of convenience, although it was for the most part rather muddy from clay and sand.

The place for the winter station being from the beginning undetermined, it might have happened that it had been fixed in a place where no, or, in any case, very few seals and penguins were to be found. Therefore the provisions taken on shore from the "Antarctic" were intended to last, without counting upon any possible supplies of seal-flesh etc., for two full years. As was however soon evident, there was a rather scanty supply of several of the most necessary articles of food, and therefore we were compelled to try to make good the deficiency. Fortunately at a distance of about 12 miles from the station there existed two colonies of penguins, and during the lighter season we also found a fair number of seals. As was the case with the other two wintering parties, seal- and penguin-meat also formed one of the principal articles of diet during the second winter. The provisions were supplied by several firms and were generally of excellent quality. The greater part of the tinned meat, soups and vegetables were supplied by Beauvais of Copenhagen and by "The United tinned-foods Factory of Sweden" of Gothenburg; the dried vegetables were supplied by Beauvais and H. Rönaasen, Christiania; the dried potatoes by the latter firm and also by C. Bödiker & Comp., Bremerhaven; the salted meat and pork was delivered by Lilljequist & Son of Gothenburg. Danish and Argentine butter and Pellerin's margarine were also amongst our supplies. The biscuits were supplied by Beauvais. Of all other articles I can here only mention a part, as dried and salted fish, salted herring, eloud-berries, cow-berries, dried bilberries (the latter are especially recommended as being both very good, easy to preserve, and requiring very little room), wheat and rye flour, beans and peas, shelled oats and barley. Of condiments we had plenty, as for instance dried onions (Beauvais), pepper, mustard and salt; these condiments proving to be invaluable when seal- and penguin-meat began to form part of our daily diet. Of lime-juice there had been taken only a little, this being principally intended to be used during sledge-trips and boat voyages. Of spirits we had brandy, Swedish punch, Bordeaux wines and also some cognac and whisky, the greater part of this being consumed during the first year. For the second year there were only very

few bottles left, which were reserved for festive occasions or for medical use.

The following tables show the consumption of the principal articles of food during the six winter-months (1st of March—1st of Sept.) of the year 1902 and for the same period during the year 1903. I give reports of consumption only during these periods, as a clearer and more uniform idea of our consumption of food can be most easily obtained from these periods; during the rest of the year some of the members were often away from the station on sledge-trips etc., and hunting was eagerly prosecuted, the spoils forming a somewhat irregular part of our diet. Of a great part of these spoils of nature, consumed during this time, I am not able to give any exact figures as to weight, *e.g.* concerning shags (cormorants), Megalestris, seal-cubs, penguin-eggs etc.

The consumption of the principal articles of provision at the winter station on Snow Hill.

	1st of March—1st of Sept. 1902		1st of March—1st of Sept. 1903	
	Total consumption for 6 men in kg.	Per man per day in gm.	Total consumption for 6 men in kg.	Per man per day in gm.
Biscuits	112.7	102.0	112.7	102.0
Hard baked rye-bread ...	80.0	72.4	—	—
Beans	46.0	41.6	18.6	16.8
Peas	20.0	18.1	40.0	36.2
Dried potatoes	43.5	39.4	—	—
Grit and maccaroni ...	28.5	25.8	36.6	33.1
Flour	150.0	135.8	380.0	344.2
Sugar	122.5	110.9	53.3	48.2
Butter and margarine ...	99.0	89.6	78.6	71.1
American bacon...	24.5	22.1	—	—
Salted meat	135.0	122.0	—	—
Salted pork	67.5	61.1	—	—
Seal- and penguin-meat	—	—	532.0	481.8
Tinned meat and fish ...	204.6	185.3	71.7	64.9
Salted herring	75.0	67.9	—	—
Salted fish	31.5	28.5	24.6	22.2
Dried fish	17.0	15.3	10.6	9.6
Condensed milk...	47.5	43.0	33.3	30.1
Cheese	30.7	27.8	—	—
Tinned vegetables ...	86.0	77.8	63.4	57.4
Dried vegetables	35.0	31.7	50.6	45.8
Dried fruit	33.5	30.3	14.0	12.6
Cloud-berries	30.0	27.1	33.3	30.1
Cow-berry-jam	30.0	27.1	—	—
Coffee (not roasted) ...	26.6	24.0	11.2	10.1
Cocoa and chocolate ...	7.8	7.0	6.4	5.7
Tea	2.5	2.2	4.3	3.8
Tinned soups	c ^a 80.0 lit.	72.4 centilit.	c ^a 22.5 lit.	20.3 centilit.

The transition from the dietary regulations of the 1st to those of the 2nd winter was made little by little during the southern summer, 1902—1903. During the last months of our stay at the station we had commenced—butter and margarine by this time beginning to run short—to use seal-blubber on a larger scale, both for the preparation of the food and as food itself. A scheme of provisioning for the following winter had been made, penguin-eggs having already been collected for this purpose, when the expedition was relieved. The eggs were partly kept in salt and partly loose in boxes, which were placed in a shady spot; they kept well. During the second summer a great number of penguins were collected; about three-quarters of these were salted, the rest being suspended, without any other preparation, on a wire on the exterior walls of the house. Here they hung the whole year, the last being used a short time before the arrival of the relief-expedition. No signs of putrefaction could ever be observed. Besides the meat, the blood, liver and kidneys of seals were also used; of shags, besides the flesh which is very good, the liver, heart and kidneys were eaten. The penguin-eggs each weighed about 120 gm.; they would certainly have been considered a delicacy even in a civilized community.

In order to give a clear view of our dietary conditions I here also produce two bills of fare, one from the winter 1902, and the other from the winter 1903, these bills of fare, too, making evident the great difference of the diet during the first and the second year at the station.

There was also consumed a great amount of other kinds of food in smaller quantities, such as pies, sardines, dried mutton, a kind of blood-puddings, tinned fruit, fruit-syrups, apple-cake and other desserts etc. With respect to the above-named figures I wish to remark, that the weight of the salted meat and pork consumed is based on the figures given by the purveyor (weight per barrel). If, however, the weight of bones, sinews etc., here included, be subtracted, I think that the net weight of the meat cannot have amounted even to half of that given. In calculating the seal- and penguin-meat, I have, on the contrary, only counted the flesh which was free from bones and also, macroscopically at least, free from fat. Thus the figures are very approximate. Still I think it is evident, that we used during both years a relatively large amount of carbohydrates; of fat, at least during the winter 1903, a somewhat small quantity, and that albuminous food, especially during the last year, entered rather largely into the rations. From the table it will also be seen, that during the first six months we lived exclusively

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
<i>Breakfast</i> (9 a.m.)	Salted herring and potatoes.	Porridge and milk.	Blood pudding and pork.	Porridge and milk.	Salted herring and potatoes.	Tinned meat and potatoes.	Porridge and milk.
<i>Dinner</i> (2.30 p.m.)	Beans and salted meat. Soup of dried fruit.	Blood pudding. Tinned soup.	Salted or dried or tinned fish. Bouillon. Dessert.	Peasoup with pork. Pancake with jam.	Tinned meat and vegetables. Soup of dried fruit.	Beans and salted meat. Chocolate soup or gruel.	Tinned soup. Tinned meat and vegetables. Dessert.
<i>Supper</i> (9 p.m.)	Pancake with jam.	Boiled dried fruit.	Laps-couse.	Laps-couse.	Porridge.	Pancake with jam.	Laps-couse.

Moreover at *breakfast* and at 5 p.m. coffee, sugar, bread and butter; at *supper* tea or cocoa, bread and butter.

Bill of fare, 22. ii. 1903—15. viii. 1903.

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
<i>Breakfast</i>	Dried or salted fish and vege- tables. Porridge and milk.	Salted meat (penguin) and vegetables.	Porridge and milk.	Salted meat (penguin) and vegetables.	Porridge and milk.	Seal-meat and vegetables.	Laps-couse of penguin-meat and vegetables.
<i>Dinner</i>	Penguin- or seal-meat and vegetables. Pancake with jam.	Blood pudding. Tinned soup. Dessert.	Penguin- or seal-meat and peas. Soup of dried fruit.	Peasoup with salted penguin. Pancake with jam.	Penguin-meat with rice or macaroni. Bouillon.	Salted or dried fish with vegetables. Chocolate soup.	Tinned meat and vegetables. Tinned soup. Dessert.
<i>Supper</i>	Cold seal-meat or fowl, tea or cocoa, sugar, bread and butter.						

At *breakfast* also coffee, sugar, bread and butter. At 5 p.m. coffee and bread, but no butter.

upon provisions brought from home, without any addition of fresh meat.

Bread was baked during the first year 2 or 3 times a week with the help of baking-powder. During the southern summer 1902—1903, however, it became evident that our store of baking-powder was not sufficient for the coming winter. By a mere chance, however, I succeeded during March 1903 in finding yeast in the last remains of our dried potatoes¹. By infecting a mixture of wheat-flour and water in a high, narrow glass pot with this yeast, I succeeded in getting good fermentation. Part of the contents of the glass pot were mixed with a large quantity of dough, which was afterwards kept at room-temperature for 24 hours, good fermentation arising during this time. The bread thus baked was at first a little sour, probably a result of a bacillus which grew abundantly in the dough at the same time. By means of repeated re-cultivations in the high narrow glass pot I have mentioned, these bacteria, however, were made to disappear almost entirely, the bread prepared with the contents of the glass pot afterwards giving an excellently fermented, well-tasting bread, having no signs at all of sourness. Afterwards the process was simplified for long stretches of time by part of a large piece of fermented dough being kneaded into the new dough, which became sufficiently fermented after 12 to 24 hours. Now and then, however, this yeast had to be purified from the increasing bacteria by cultivation in the glass pot. Bread fermented in this manner was baked each day of the last winter at the station.

Regarding personal cleanliness it must be mentioned that bathing, *i.e.* washing of the whole body with hot water and soap, had been ordered to be done every 3rd or 4th week, an order that was, as a rule, well observed by the members of the station.

At the winter station the state of health was during the whole time very satisfactory. Now and then, of course, there were complaints of some ailments of a transitory and not at all dangerous nature, such as muscular-rheumatism, colic, diarrhoea, dyspepsia, etc., etc., none of the party being however ever compelled to take to bed for 24 hours at a time. A few cases may here be mentioned which might easily have taken a grave turn. One of the members of a long sledge-trip, October 1902, over-exerted himself during the journey. On returning to the station, after a very long and trying march and exposed to a full storm,

¹ Though a rather rich bacterial flora existed in the superficial layers of the earth of Snow Hill, I never found any yeast there.

this man became unconscious for about 5 minutes, the heart being at the same time considerably dilated with a systolic murmur over the apex; the pulse being rapid, unequal and intermittent. At the same time the patient was snow-blind in both eyes. He recovered completely however. Another of the members was taken ill with Otitis media. After treatment by Politzer's method etc., the symptoms fortunately disappeared. A great many insignificant external injuries, such as contusions, burns, frost-bites and snow-blindness also occurred. No really severe case of snow-blindness ever occurred, we being soon induced by experience to use snow-glasses, when the light out of doors was such that snow-blindness was to be feared. As has been noticed by several other polar expeditions, we too observed that the greatest danger of snow-blindness was not when there was bright sunshine and clear air, but when the atmosphere was misty or when a low fog made the sunlight spread diffusely, thus obliterating all shadows. As with the other parties, sleep and appetite were generally excellent. An important fact is, that none of the wintering parties suffered from any of the common forms of "colds," such as catarrhs of the nose, larynx, trachea and bronchi, articular rheumatism etc., and this in spite of the numerous occasions (if compared with ordinary conditions) that occurred for the contraction of such "colds." As with the other parties, instances of irritable bladder were also occasionally observed at the winter station, though not so generally and regularly as in the other parties. As nothing pathological was observed, and nothing abnormal could be noticed in the urine (the quantity was, perhaps, a little increased), I am inclined to attribute these symptoms to certain dietetical conditions, having myself observed that such desire to urinate set in especially after eating the liver and, possibly, the kidneys of seals. These organs were often used by the two other parties, but more seldom at the winter station, circumstances which may perhaps explain the difference in the occurrence of the symptoms. Probably the cold and a certain degree of increased nervous irritability contributed to excite the symptoms mentioned.

As regards psychical conditions we have to note a certain degree of depression and increased irritability, exhibited by the greater part of the members of the three wintering parties, especially during the dark season; these disturbances in no case, however, taking the form of melancholia or any other mental disease.

The members of the party at Snow Hill were usually weighed every fortnight during the stay at the station. As shown by the adjoined

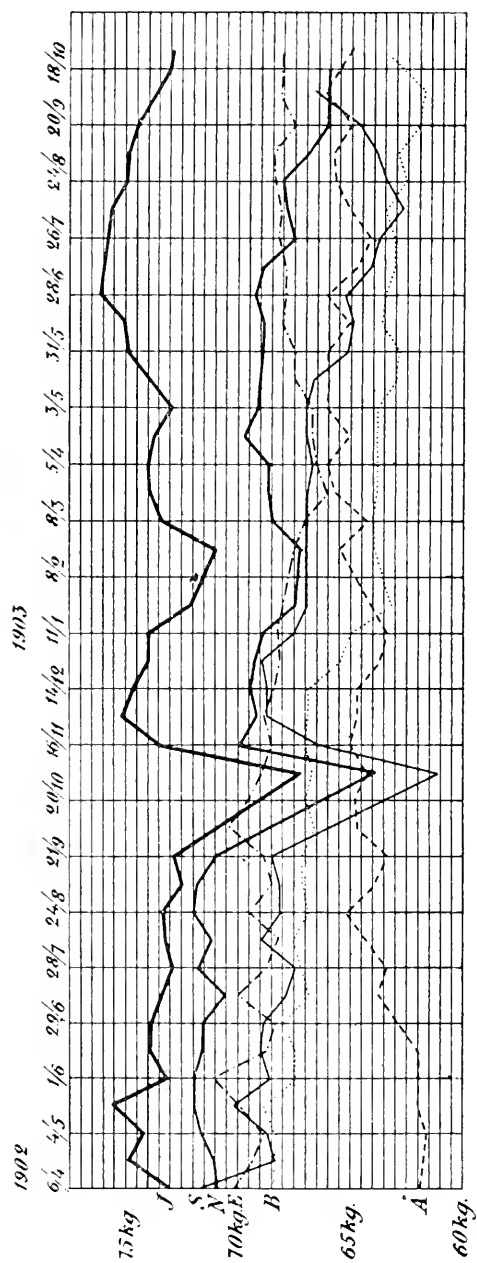


Fig. 5. Curves of the weights of the persons at the station on Snow Hill.

weight-curves the greater part showed a very gradual diminution in weight. The most typical feature in the weight-curves is a distinctly marked falling during the summer (Dec., Jan., Febr.). The curve Å refers to a young man of 18, who grew rapidly during his stay at the station, this causing the incongruity of his curve. The sharp sinking of the curves J, S, and N during the month of October 1902 was caused by a sledge-trip, performed during this time by the persons in question. In order to show the rapidity of the increase of weight, which may take place after a decrease due to severe bodily exertion, I give the following figures :

Persons	4. xi. 1902 8 a.m.	4. xi. 1902 11 p.m.	5. xi. 1902 11 p.m.	16. xi. 1902 10 p.m.
N.	63.9 kg.	68.0 kg.	69.2 kg.	69.8 kg.
J.	67.2 „	70.8 „	—	73.3 „
S.	61.1 „	65.0 „	67.0 „	66.4 „

The blood of the persons at the winter station was occasionally examined during the whole period of their stay; the percentage of haemoglobin and the number of red corpuscles were never lower than under normal conditions; on the contrary, changes in the other direction developed themselves during the 2nd winter, the blood becoming deeper coloured than normal and the number of red corpuscles increasing to 6,000,000 per cubic mm. during the latter part of our stay on Snow Hill. Microcytes were often present, but normo- or megalo-blasts never occurred. Simultaneously there was observed a considerable diminution in the absolute number of the white corpuscles, a total of only 4 to 5000 per cubic mm. being not seldom met with. A detailed account of these haematological analyses, as well as of the bacteriological researches which were made during the whole of my stay at the station, will be published later.

6. *The return of the expedition, 11 Nov. 1903—10 Jan. 1904.*

The expedition returned on board the Argentine relief-vessel, the gunboat *Uruguay*, which arrived at Buenos-Ayres on the 2nd of Dec. 1903. On the 10th of the same month we left this city on board the German steamer *Tijuca*, arriving at Hamburg on the 7th of Jan. 1904,

and at Stockholm on the 10th of the same month, after an absence of $2\frac{1}{4}$ years from Sweden. During the voyage home there occurred no grave cases of illness. We need only notice a case of gonorrhoea (acquired in Buenos-Ayres), this being the sole case of venereal disease during the whole expedition. During our stay in Buenos-Ayres and in the tropics there occurred several slight cases of diarrhoea. There were also some complaints during this time of rheumatic muscular and neuralgic affections.

I wish to draw some conclusions regarding the influence of food upon health during polar-expeditions; conclusions to which I have been led, partly through experience of this expedition, partly through the study of what has been observed regarding these matters during many other expeditions. That the food is the determining factor as regards outbreaks of scurvy during polar-expeditions, sea-voyages, sieges etc., has long been acknowledged, but we are still in ignorance concerning the particular fault in the food which is the ultimate cause of the disease. Without entering upon any detailed criticism of all the different theories in the matter, I will here merely cite the one propounded by Mr Sophus Torup, Professor of Physiology at the University of Christiania, as it is in my opinion the only one giving an intelligible explanation which, in its leading features at least, is compatible with the actual circumstances. The essential principle which distinguishes Torup's theory from those commonly adopted is his not assuming the cause of the foods producing scurvy to be absence in this food of certain elements existing in common fresh food and indispensable for the body, but, on the contrary, that the cause of disease is to be ascribed to the *introduction into the body of some non-organized matter, foreign to, and dangerous for the human organism*. After having examined what has been written on the subject Torup has arrived at the conclusion, that "scurvy must be caused by a kind of poisoning: that, by a peculiar and slow decomposition, taking place in badly preserved (e.g. badly salted) meat and fish, there are formed poisons, nearly related to the ptomaines, through the continued consumption of which the disease is created." (*Over the Polar Sea*, by F. Nansen, Swedish edition, Part 2, p. 566.) I wish, however, at once to remark that this theory cannot be true in its entirety, there being unquestionable examples of expeditions having been attacked by scurvy, where, as far as can be judged, the provisions have been of first-rate quality, and the blame cannot be ascribed to *bad* preservation of the food. To be able to show the legitimacy of the above-mentioned

theory, it would also be necessary amongst other things to quote particulars from a great many expeditions, a procedure which cannot now be attempted. It is enough to state that having examined the accounts of these voyages, as regards the conditions of the food and the state of health, I have ascertained that scurvy (the typical form) has broken out only during expeditions using preserved animal food (salted, dried, or tinned); the disease having broken out, whether food of so-called antiscorbutic qualities (*e.g.* dried and tinned fresh vegetables, juices of fruit, potatoes, tinned unsalted meat, perfectly fresh meat, etc.) was issued simultaneously or not.

As an example of such an expedition, where, in spite of varied and abundant provisions, in which there cannot be any question of want of different nutritive elements, salts, etc., scurvy has still broken out, I will only refer to the Austro-Hungarian North-Polar-Expedition of 1872–1874; where scurvy broke out, in spite of the presence in the food of, amongst other things, a rather abundant supply of tinned vegetables, lime-juice, tinned unsalted and even perfectly fresh meat etc. etc.; salted meat however formed part of the diet. Similar conditions existed in the case of the Swedish Expedition to Spitzbergen under Nordenskiöld, 1872–1873; in this case 41·7 % of the whole party being attacked by scurvy. Although there was a great variety in the provisions, the supply was here, however, a little scanty.

On the other hand, several instances can be found of expeditions escaping entirely from scurvy in spite of extremely unfavourable hygienic conditions, the provisions consisting, however, entirely, or nearly so, of fresh meat (obtained by hunting). Such, for instance, was the case during the wintering of Nansen and Johanssen on Frans-Joseph Land; here they lived for 9 months solely upon the meat and blubber of ice-bears and walrus, and during the whole time were in perfectly good health; this fact being of itself sufficient to exclude the theory that want of vegetables is the cause of scurvy. To this example I think I might also add that of two of the wintering parties of the Swedish Antarctic Expedition.

The conviction that the cause of scurvy lies in the consumption of salted meat and fish (possibly also the lack of fresh vegetables) has led to a more or less total provisioning of expeditions etc. with tinned meat and vegetables, under the belief that the possession of these tinned foods constituted an unfailing guarantee against the outbreak of scurvy. Even if this should be the case as regards scurvy (the typical form), experience, however, has taught us, that other patho-

logical symptoms (these possibly nearly related to scurvy) might arise with such a diet, the results being quite as fatal as those of scurvy. We have a very recent example of this in the Belgian Antarctic Expedition under de Gerlache 1898–1899. The question of provisioning with tinned foods being of the greatest practical importance and the condition of things during the aforesaid Belgian expedition being a good illustration of this, I will take the liberty of dwelling a little on the voyage.

The Belgian expedition wintered on its vessel, the “Belgica,” in the ice between 70° and 72° S. lat., corresponding to a polar night from the 15th of May to the 22nd of July. The greater part of the provisions consisted of tinned fresh foods (meat, fish and vegetables), supplied by well-known large tinned-food firms (Belgian, French and Norwegian). During the winter all the members of this expedition were taken ill, the symptoms being in every case very similar; one died and several nearly met with the same fate. I shall now give some extracts from the description of the journey, made by the surgeon of the expedition, Dr F. Cook (*Through the first Antarctic Night*, London, 1900). Unfortunately however the data of interest from a medical point of view, given in this account, are rather defective and unscientific.

As early as the 31st of May Dr Cook writes: “We became pale with a kind of greenish hue; our secretions were more or less suppressed. The stomach and all the organs were sluggish and refused to work. Most dangerous of all were the cardiac and cerebral symptoms. The heart acted as if it had lost its regulating influence. Its action was feeble, but its beats were not increased until other dangerous symptoms appeared. Its action was weak, irregular and entirely unreliable throughout the night. The mental symptoms were not so noticeable. The men were incapable of concentration and unable to continue prolonged thought. One sailor was forced to the verge of insanity, but he recovered with the returning sun....” June 3rd: “We are all eating appreciably less now than during the bright season—and either there is a constant inclination to sleep or persistent insomnia. There is much indigestion now,—fermentation, gastric inertia, intestinal and gastric pains, imperfect hepatic action, and a general suppression of all the digestive secretions. The heart is unsteady, easily disturbed and mitral murmurs, which I have not heard before, are audible. Temperatures, almost without exception, are subnormal. The breathing is often difficult, the blood retreats from the skin, but the larger veins are abnormally full. Piles, haemorrhoids, headache, neuralgia, rheumatism,

are the systematic complaints." June 19th : "Anaemia, or a condition allied to it, in one form or another and under various descriptions, is always found if sought for by an experienced eye. This malady we have had in by far the severest form which I have noticed in any Arctic experiences, and more severely than is recorded in the literature of polar exploration. We have lost one officer, and a second barely escaped death. The marines are all afflicted; the condition is truly alarming," etc. On July 12th it is reported that the second in command, who had remained quite well until this date, after having made some scientific observations out of doors, had, on leaving the open air, suddenly been taken ill. "But to-day I have to record the saddening news that L. is suddenly failing. Not that he has complained of any ill feeling, for he still maintains that he feels well; but in the usual daily examination I notice that his pulse is intermitting, the first sign of coming debility. He is assuming a deathly pallor, does not eat, and finds it difficult to either sleep or breathe. There is a puffiness under the eyes, his ankles are swollen, and the entire skin has a dry, glossy appearance." Dr Cook then speaks about the treatment of this form of "polar anaemia" (analyses of the blood, however, have not been made, as far as I can see). Several remedies were used, producing, however, no effect or but a temporary one; thus iron and arsenic were given. By and by these remedies were given up as being totally inoperative. "Fresh food, artificial heat, a buoyant humor, judicious clothing and the least possible humidity are the conditions which suggest a rational treatment. The plan of treatment in brief is as follows: As soon as the pulse becomes irregular and rises to 100 beats per minute, with a puffiness of the eyes and swollen ankles, the man is stripped and placed close to a fire for one hour each day. I prohibit all food except milk, cranberry sauce, and fresh meat, either penguin- or seal-steaks fried in oleo-margarine. The patient is not allowed to do anything which will seriously tax his heart. Laxatives are generally necessary and vegetable bitters with mineral acids are a decided help. Strychnine is the only remedy which has been of service in regulating the heart, and this I have used as routine."

The patient just named (L.) was treated in this manner and recovered after about 2 weeks' treatment. On the 15th of July we read: "We are still very feeble. An exercise of one hour sends the pulse up to 130, but we have all learned to like and crave penguin-meat." 17th of July: "If we had not fresh meat to eat and an abundance of fuel to give heat, I am sure we would have an alarming mortality in less than a month. Several lives have certainly been saved by eating penguins,

and we shall always owe them a debt of gratitude." The writer further states that alcohol, until the outbreak of this disease commonly used in the form of wines to the dinner, could no longer be borne, the action of the heart being disturbed by its employment. Nothing in the external appearance of the patients indicated the sickness with the exception of the aforesaid paleness; no decrease in the weight of body was observed during the winter. By and by the whole party recovered, seal- and penguin-meat having been almost exclusively introduced into the diet instead of the tinned meats.

It is thus seen that the disease was characterised by several symptoms, disturbances of digestion and feebleness of heart being the chief amongst them; in the case of some persons the disease even showed itself solely through symptoms affecting the heart. (Compare this disease with the special form of scurvy called the "pure scorbutic anaemia.") The cause of the disease indicated in the above account probably was some sort of poisoning, by the tinned meats. These being carefully sterilized and kept in hermetically closed tins, the supposition at once offers itself, that the poisons in question had been formed by autolytical processes, a conclusion which, to my mind, ought also to be applied to the poisons present (according to Torup) in certain salted meats and fish, the consumption of which is said to have resulted in scurvy.

I will only adduce one more example, to which however I attach the greatest importance, *viz.*, the recently returned British Antarctic Expedition (which operated simultaneously with the Swedish one), an expedition where, as well known, no expense had been spared, all modern resources being utilized. Unfortunately I have not been able to get more particulars than those given by the leader of the expedition, Commander Scott, in the July number 1903 of the Royal Geographical Journal. Here it is mentioned, that during the winter of 1902 the whole party had been attacked (though slightly) by a disease that was "undoubtedly scurvy." During the winter fresh seal-meat three times and fresh (?) mutton once a week had been added to the dietary in addition to the provisions brought from home. About one month before the outbreak of the disease the stock of seal-meat had begun to run short, the fresh meat being thenceforward given not so often as before. The measures which were here taken, and with the help of which everybody soon recovered, were the following: all tinned meats were laid aside, fresh seal-meat being given instead; and extra rations of tinned fruit were distributed and lime-juice prescribed. In addition the holds and

store rooms were thoroughly overhauled and disinfected. After the outbreak of the disease the contents of the tins were always examined by the surgeon of the expedition before being used; but nothing remarkable could ever be observed.

Whatever may have been the origin of the above-named cases, still it seems to be a fact that grave disease may arise as a consequence of the consumption not only of salted, but also of tinned unsalted, meats and fish. Furthermore these diseases have proved avoidable (or curable) by the eating (as far as regards animal food) of fresh meat exclusively or nearly so. Fortunately nature nearly everywhere in the Arctic and Antarctic regions, and especially during the light season, offers an abundance of game, from which expeditions may, without any great difficulty, procure stores of meat for the winter, the climate and the bacterial conditions being such that no difficulty exists as to the preservation and keeping of the food; and, finally, it has been proved that the food, in this way derived from the polar animals, is not at all repugnant to the taste, such an idea being quite an unjustifiable prejudice, which has gradually died out during the expeditions of the last few years. In consequence of what has here been stated I take the liberty of proposing the following principles to be followed as to the provisioning of polar expeditions (partly also applicable to provisioning under other circumstances):

A complete provisioning with vegetable as well as with animal food ought to be made before the starting of an expedition for the polar regions. The utmost caution must be observed when choosing the provisions, especially the animal food: this should be taken only from well-known firms. The greater part at least of the tinned meat and fish ought to consist only of such kinds of food as have proved to be harmless during previous expeditions. Further, I consider that it may be of the utmost importance that the *tinning and the salting of meat and fish be undertaken as late as possible* before the starting of the expedition, in order to escape in the greatest possible degree autolytical poisons which may possibly arise; as autolytical processes continue, as far as is known, for long periods (it may be several years)¹, I think it may also be assumed, that out of two similar barrels or tins of meat or fish, in which autolytical processes are going on, the autolytical

¹ These opinions of mine as to the influence of time and temperature upon the autolyses are founded upon examinations made by Dr S. Schmidt-Nielsen of Christiania. ("Ueber den Reifungsvorgang beim Pökeln von Häringen," *Det Kongl. Norske Videnskabers Selskabs Skrifter*, 1901, No. 5.)

products are found more abundantly in those prepared earlier than in those prepared later on. The animal provisions ought also always to be kept in a cold place, upon ice, if possible, analyses having proved, that autolytical processes go on considerably more slowly at lower temperatures than at higher.

A large amount of condiments ought also to be brought, especially dried onions, pepper, mustard and, of course, salt. Dried vegetables and potatoes have proved to be excellent to keep, easily preserved, and they occupy little space.

All tinned or salted animal food ought to be considered principally as provisions in reserve. In the polar regions provisioning with meat for the coming winter ought to be made in good time. The greater part, at least, of this meat should be kept unsalted and unprepared, either suspended in the open air in a shady place, for instance on the walls of the house or the sides of the ship, in the rigging etc., or buried in the snow. The condiments we have just mentioned are intended to give variety to the dishes prepared from this meat. The preserved animal foods should be used only exceptionally and as a variation in the diet, or if the stock of fresh meat, collected on the spot, should turn out to be insufficient.

The Map accompanying this paper (p. 512) appeared in *The Geographical Journal* for February, 1904.

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¹ The size of the publication is given roundly in centimetres.—Ed.

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[For particulars address the Secrétaire Général, Prof. Miguel Bombarda, Lisbon.]
- The Journal of Experimental Zoology.* Edited by W. K. Brooks, W. E. Castle, E. G. Conklin, C. B. Davenport, R. G. Harrison, H. S. Jennings, J. Loeb, T. H. Morgan, G. H. Parker, C. O. Whitman, and E. B. Wilson. Vol. 1., No. 1, May, 1904. Baltimore: *The Journal of Experimental Zoology*, N.E. cor. Wolfe and Monument Sts., Baltimore Md., U.S.A. (Price for subscribers in the United States, Canada and Mexico, \$5, in other countries \$5.50.) 25 × 17 cm.
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